MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. LII MARCH-APRIL, 1960 No. 2

CONTENTS

[MYCOLOGIA for January–February, 1960 (52: 1–170) was issued June 2, 1961]

PUBLISHED BIMONTHLY FOR

THE NEW YORK BOTANICAL GARDEN

AT PRINCE AND LEMON STS., LANCASTER, PA.

Second-class postage paid at Lancaster, Pa.

MYCOLOGIA

Published by

THE NEW YORK BOTANICAL GARDEN

IN COLLABORATION WITH THE

MYCOLOGICAL SOCIETY OF AMERICA

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HANSENULA HOLSTII, A NEW YEAST IMPORTANT IN THE EARLY EVOLUTION OF THE HETEROTHALLIC SPECIES OF ITS GENUS

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(WITH 1 FIGURE)

Previous to 1951 the known species of *Hansenula* consisted of yeasts living in soil and water and on fruit where they competed with a wide variety of other microorganisms. In 1951 some additional species were reported that live on the sap of trees and depend upon bark beetles for transportation from the cambial layer of one tree to another. The species associated with broadleaf trees are more primitive than the species found in soil and water; the species that live under the bark of conifers are the oldest now known. The earliest evolved members of the genus evidently were haploid species, one homothallic and the other heterothallic; from each of these primitive ancestors apparently two lines of species evolved.

The two ancestral species were similar or identical to *Hansenula capsulata* Wickerham (**1951**), the homothallic ancestor, and the new species *Hansenula holstii*, its heterothallic counterpart. The former produces no hyphae; the latter does. The two differ also in their biochemical reactions.

Each of the two species gave rise to two phylogenetic lines (FIG. 1) in which the dominant evolutionary characteristic was an increasing

[1] With the technical assistance of Kermit A. Burton and Jane A. Roberson.

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tendency for each succeeding species to live as a mixture of haploid and diploid cells, with the ratio of diploid cells increasing. The number of diploid cells produced in *H. capsulata* and *H. holstii* is so infinitesimally small that the species, so far as known, are never isolated from nature in this form. The diploid is isolated only with difficulty in the laboratory. About midway up these phylogenetic lines, which led to a free-living existence in soil, water, and fruit, the ratio of diploid to haploid cells is so large that either form can be isolated readily from nature or in the laboratory. In the most recently evolved species of both the heterothallic and homothallic lines, all or nearly all of the vegetative cells are diploid.

Numerous secondary evolutionary characteristics also indicate the level of evolutionary progress reached by each yeast in the two chains of species developing independence from trees. The most primitive species have small, encapsulated cells that give the colonies a somewhat watery, mucoid consistency and appearance; they require a maximum of vitamins from their environment; they produce thin pellicles, if any, on liquid media and no esters; sexual reactions are weak, especially in the heterothallic line. Intermediate species produce larger cells, the colonies are butyrous and glistening, they require fewer vitamins, they ferment more sugars and the fermentations are stronger, esters are produced, and sexual reactions are increasingly stronger. The most recently evolved species produce the largest cells; the colonies are butyrous and mat in appearance because the top layer of cells are hydrophobic. Such cells float on the top of liquids as thick pellicles thereby stimulating the species to develop strongly the ability to oxidize alcohol and organic acids. These compounds are produced within the liquid by cells which do not possess floating principles. As many as five sugars are fermented strongly with ester production. The latest evolved species synthesize all of the vitamins needed for growth, and their sexual reactions are generally strong.

In contrast to the homothallic and the heterothallic evolutionary lines leading to independence from trees, there are two other sexual lines starting from *H. capsulata* and *H. holstii* that lead to greater dependence upon bark beetles and coniferous trees. The species gradually lost the ability to cause a gaseous fermentation of sugars until now fermentative capacity has completely disappeared. No strongly oxidative (heavy pellicle-forming) species were produced. Vitamin requirements remained at a maximum. The rate at which the completely diploid state was approached was evidently slow, and even in the most recently evolved species sexual reactions are often weak. One species, *Hansenula*

wingei Wickerham (**1956**), counteracts the trend toward weaker sexuality by a novel mechanism, exceedingly efficient, that stimulates zygote formation. Zygote formation in strains lacking this new biological process, called sexual agglutination, is at a minimum. Strains possessing sexual agglutination produce zygotes faster than all species of yeast not possessing it. From this mechanism one gets a glimpse of evolution in action.

Primitive heterothallic haploids of *Hansenula* have weak sexual reactions, and it is necessary to demonstrate sexual reactions in such species before they can be properly placed taxonomically. It is of value to indicate the difficulty met in obtaining sexual reactions in *H. holstii*, and to present methods by which the sexual reactions were made so strong that their ready demonstration in derivatives of the type strain is now a simple matter. The media used and the basic procedures for obtaining sexually reactive haploids from nature, as well as obtaining mating types from sporulated diploid species, have been given in detail by Wickerham and Burton (**1954a and b**).

EXPERIMENTAL RESULTS

H. holstii is commonly found in frass of a number of genera of coniferous trees. The species is less commonly found in gums exuded by some trees of the genus *Prunus* (nomenclature of trees according to Little, **1953**). Because this yeast is heterothallic and almost exclusively haploid, ascospores are never produced by isolates picked from single colonies. Immediately following the discovery of haploid mating types of *Saccharomyces lactis* Dombrowski, *Endomycopsis ohmeri* Etchells & Bell and *Endomycopsis chodati* Wickerham & Burton in nature (Wickerham and Burton, **1952**), it became obvious that the apparently non-ascosporogenous, nitrate-positive, mucoid, weakly fermentative yeast from frass and gums of trees could be a primitive heterothallic species of *Hansenula*. An attempt was made to obtain ascospores by mixing isolates on sporulation medium in combinations of 4 strains in hope that some mixtures would contain opposite mating types that would conjugate and produce ascospores. Consequently, as isolates were obtained, they were mixed in groups of 4 on slants of malt-extract sporulation-medium and incubated for long periods of time at 25° C, but no conjugations and no spores were observed. After 46 strains had been isolated from coniferous trees and 9 from gums of peach and wild cherry trees, a comprehensive experiment was set up. Twelve mixtures of 2 or 3 strains, each mixture consisting of strains from the same individual tree, were made; 11 mixtures of groups of 4 strains from the same geographic area but

not from the same tree were made; and 11 mixtures by fours were made, each mixture consisting of strains from widely different areas and different species of trees, so far as possible. All 34 mixtures failed completely to form spores.

In November 1950, Mr. H. P. Dussault, bacteriologist of the Gaspé Fisheries Experimental Station, Grand River, Province of Quebec, Canada, sent us some samples of frass taken from under the bark of slabs from saw logs. When two typical strains of the species, isolated respectively from frass labeled by Mr. Dussault as yellow spruce (*Picea rubens*?) and white spruce (*Picea glauca*?) were mixed, they conjugated and produced hat-shaped ascospores. As spores of primitive species of *Hansenula* are prone to do, they broke free from the asci, grew large and refractile yet maintained their characteristic shape, and lost their acid-fastness.

TABLE I

RELATIONSHIP OF SEXUAL ACTIVITY TO SOURCE OF CULTURE

Source	Number of strains tested	Number of strains reacting	Percentage of strains reacting
Pinus	84	1	1
P. engelmanni	8	0	0
Picea, except *P. engelmanni*	5	3	60
Larix	4	2	50
Juniperus	2	2	100
Abies	2	0	0
Pseudotsuga	1	1	100
Prunus	9	6	67
Totals	115	15	13

The two opposite mating types of *H. holstii* were given numbers NRRL Y-2154 and Y-2155. Each was mixed with each of the 55 strains used in the previously mentioned experiment that had been entirely negative. Strain Y-2154 did not react with any. Strain Y-2155 mated with 5 of the 9 strains from cherry- and peach-tree gums (all from the vicinity of Marion and Peoria, Illinois) and with 5 of 46 strains from conifers. The maximum sporulation after 3 weeks of incubation was an estimated 2 to 3 per cent. As other strains became available, they were mated with Y-2154 and Y-2155, and with more reactive inbred mating types, which will be described presently. As noted in TABLE I, although most strains of *H. holstii* were isolated from frass of species of *Pinus*, only one of 84 strains from this source reacted sexually. It conjugated with one of the inbred mating types, but failed to produce ascospores. Eight strains were isolated from *Picea engelmanni* and

none were sexually active. Among the few isolates from other species of *Picea*, and from *Larix*, *Juniperus*, *Pseudotsuga*, and *Prunus*, the ratio of sexually active strains is much higher. The incidence of *H. holstii* in these last mentioned genera may be higher than our figures may infer, because we did not have as many frass or gum samples from them as from *Pinus*.

Of the 15 sexually active strains found, 12 are of the same sex as Y-2154, and 3 are of the same sex as Y-2155. Of these 3, 2 mate only with inbred mating types. Conjugations are produced but ascospores are not. The third is Y-2155, sexually the most potent of all the mating types yet isolated from nature, and the only one of its sex that has mated with other strains and produced ascospores. Thus, if strain Y-2155 had not been isolated from frass from the Gaspé Peninsula, sexuality for this species would still be unknown. If a natural isolate is found as sexually potent as Y-2155 but of the opposite sex, distribution of sexually active strains among our isolates probably would be more nearly equal in number. One might hope that by mating Y-2154 and Y-2155 and then isolating ascosporic cultures, some would have the strong mating ability of Y-2155, but be of the same sex as Y-2154.

Mating type Y-2154 constantly produces smooth, highly glistening, mucoid colonies. The capsular material is a phosphorylated mannan which has interesting properties that may lead to practical applications (Jeanes *et al.*, **1958**). Y-2155 occasionally yields nonmucoid colonies, and strain Y-1864, isolated from gum of a peach tree in Peoria, gives rise to colonies that are not merely nonmucoid but strongly rugose as well. Y-1864 is of opposite sex to Y-2155.

Variants may be obtained by growing single giant colonies in the center of petri dishes for 30 to 40 days at 25° C. Sectors of diverse appearances are common at the edge of the colonies, and cells from them may be used to start a second generation of giant colonies. Some of the latter may be conical, butyrous, smooth, and glistening; others spreading and rugose, yet glistening; and still others may be highly filamentous and mat. Frequently the second serial giant colony will not be similar to the sector from which it arose, but will be entirely mucoid like the parent culture, and like it, may drip onto the cover of the petri dish.

It was thought that capsules might hinder conjugation. Therefore mixtures were made of Y-1864 and Y-2155 in various combinations, the cells being taken from mucoid or nonmucoid growth on 2-day-old petri dishes. The cells were mixed on malt-extract (ME) sporulation slants which favor growth in the mucoid state. When the cultures were ob-

served microscopically after 17 days at 25° C, the percentages of spores to vegetative cells presented in TABLE II were counted.

This experiment suggests that cells with capsules, which are reduced or absent, produce more ascospores than strongly capsulated cells. A lack of capsules on appropriately grown cells of Y-2155 does not solely account for its superior sex potency however, because the noncapsular form of Y-1864 cannot mate with any strains other than Y-2155, including inbred strains.

TABLE II

INFLUENCE OF CAPSULES ON PRODUCTION OF ASCOSPORES

Inoculum	Ascospores, per cent	Growth of mixed mating types on malt-extract slant
Y-1864 mucoid + Y-2155 mucoid	1.8	Mucoid
Y-1864 not mucoid + Y-2155 mucoid	1.2	Mucoid
Y-1864 mucoid + Y-2155 not mucoid	5.1	Butyrous and slightly rugose
Y-1864 not mucoid + Y-2155 not mucoid	8.4	Rugose and pitted

It is believed that greater zygote formation is incidental to, and not the main reason for, the reduction of capsular material. More likely, temperature changes and variations from anaerobic to aerobic conditions are responsible for the loss or gain of capsules in some strains of the species. The long tunnels of bark beetles may be rather low in oxygen, since many microorganisms grow along the walls. As the cultures isolated are mucoid, this form may be regarded as somewhat anaerobic. The rugose, nonencapsulated type is relatively hydrophobic and autoagglutinative—properties which favor the development of pellicles on liquid media—whereas the encapsulated form is hydrophilic and nonagglutinative—these properties favor growth as sediment. The mat colony in *H. holstii* probably is a weak expression of a tendency to develop into a more aerobic type; generally, this tendency is increasingly stronger in the more recently evolved species.

H. holstii, like some other frass yeasts, produces a small percentage of vegetative cells which usually bear one long, narrow, pointed, tapering appendage. It may be many times the length of the cell proper. The appendage is not observed in all cultures. Generally, tapering or sharply pointed vegetative cells and ascospores are rather commonly believed to aid in dissemination by insects, and this may be the case here. However, it was also thought the tapering appendage might serve to pierce the capsule of a cell of opposite sex and thus act as a conjugation tube. Microscopic observations of sporulated mixtures have not supported this theory.

A sporulated mixture of Y-2154 and Y-2155 was heat-treated at 58° C to kill the vegetative cells. Plates were streaked at intervals, and colonies developing on the 10- and 12-minute plates were restreaked for purity. Colony isolates were selected and mated separately with parent strains Y-2154 and Y-2155. Four failed to mate with either parent. Five produced from 0.1 to 5 per cent spores, and one produced about 30 per cent spores at 8 days. This exceptional strain, ascospore isolate No. 1, more completely designated as (Y-2154 x Y-2155)-1, on repeated matings with Y-2155 produced from 30 to 50 per cent ascospores at 8 to 20 days. Isolate No. 1 has a much greater tendency to exist in the nonmucoid state than its parent Y-2155.

Ascosporic isolate No. 1 was mated with parent Y-2155 and the sporulated mixture was heat-treated. Sixty-three ascospore isolates were picked, and every one mated and produced ascospores with either Y-2155 or ascospore isolate No. 1, depending upon its sex. Several isolates of both mating types, when mated to the parent cultures and to each other, produced 20 to 30 per cent ascospores in 13 days. None of the most actively sporulating isolates showed self-diploidization when placed individually on sporulation medium.

Four of the best sporulating ascospore isolates of one sex were grown separately and then mixed together. Portions of this mixture were mixed on sporulation slants with each of 75 natural isolates, most of which had been tested previously with strains Y-2154 and Y-2155 and found negative. Each of these 75 natural isolates was also mixed with a mixture of 4 of the best sporulating isolates of the other mating type. The cultures were incubated 19 days and then examined. Only 2 strains reacted. Both had been isolated from frass from Germany, one from *Larix europaea* and the other from *Pinus sylvestris*. They produced conjugations only. Both were of the same sex as Y-2155, yet neither mated with natural isolate Y-2154. Thus the highly sporulating, inbred-mating types do not react with substantially more natural isolates than do the two original natural isolates from which the inbred mating types were obtained, nor is the sporulation any stronger than customarily obtained with the original parents.

In 1956, Dr. Lodder of Delft studied for a few months at our laboratory under a grant from the American Association of University Women. One of her experiments was concerned with the very weak sexuality observed in primitive heterothallic haploid species of *Hansenula*, and the increased sporulation that might be obtained through inbreeding. She mated Y-2154 and Y-2155, heat-treated the sporulated cultures, and mated the ascosporic isolates thus obtained with the par-

ents. Isolate (Y-2154 x Y-2155)-118, redesignated Y-2448 for convenience, showed ascospores and no conjugations in both mixtures, thus indicating the isolate was diploid. Its cells, but not its colonies, are considerably larger than those of its parents; these conditions are typical of haploids and diploids of primitive yeasts. The diploid constantly produces capsules and sporulates rather slowly, producing few spores at 7 days, but as many as 60 per cent at 26 days. The asci are not conjugated, of course, but consist of a single cell usually containing two, rarely three, hat-shaped ascospores. The carbon assimilation and fermentation reactions are typical for the species. The diploid is bisexual, having arisen from conjugation of ascospores of opposite sexes or their progeny after the heat-treatment plates were streaked. The zygote reproduced by budding rather than forming ascospores. When heat-treated, the sporulated diploid yields both sexes, some of which mate with natural isolates Y-2154 and others with Y-2155. The diploid is easily maintained pure by lyophilization, retaining its ability to produce ascospores. All cells remain diploid so long as the culture is not allowed to sporulate. If sporulation does occur, the culture may be streaked, then colonies are selected. Each colony is transferred to two slants, the one being immediately refrigerated while the other is tested for ability to produce ascospores. All which produce ascospores came from diploid colonies. The refrigerated culture corresponding to the one producing the most ascospores is kept as the vegetative diploid stock. It will have produced no ascospores during the short time it was held in the refrigerator.

DESCRIPTION OF *HANSENULA HOLSTII*

H. holstii has been repeatedly isolated from frass of coniferous trees of North America, Europe, and Asia, and more rarely from soils and streams of coniferous forests. It has been found in gums of *Prunus*, principally from wild black and red cherries (*Prunus serotina* and *P. pensylvanica*) and unsprayed peach trees. It was absent in gums from several sprayed peach trees.

Colonies are usually mucoid, more watery than viscous, often with a fringe of hyphae that develop as the colony ages. Pseudohyphae and true hyphae up to 2.5 mm in length are produced, and well-differentiated blastospores are formed singly, in pairs, or as short chains on the hyphae. The blastospores are usually ellipsoidal to short cylindroidal, occasionally spheroidal and ovoidal, and may end in long pointed tapers from one to several times the length of the cell. The blastospores on the hyphae vary from 1.7×2.6 to $3.4 \times 6.0\,\mu$, while those at the edge of a young

nonfilamented colony may vary from 0.9×1.7 to $3.4 \times 7.2\ \mu$. These extremes are not seen in most strains. Pellicles are seldom formed on liquid media and the pellicles are very thin.

Sexually active strains are seldom encountered among strains from *Pinus*, but evidently are commonly found in gums of *Prunus*. Sexual reactions between opposite mating types from nature are weak, but may be increased by backcrossing. Bisexual diploids are not isolated from nature but may be isolated in the laboratory. Ascospores of the conjugated haploids are hat-shaped, 2.0 to 2.5 μ in diameter, and the maximum is apparently 2 per ascus. The ascus breaks early and liberates the ascospores. The spores enlarge reaching 3.4 μ, become refractile, and lose their acid-fastness. The vegetative cells of the diploid are larger than those of the haploid, and the ascospores of the diploid are larger than those of the haploid in which nuclear fusion occurred just prior to sporulation. Asci containing 3 spores are occasionally observed in the diploid, but the usual number is 2.

The most decisive characteristics for identifying the species are its biochemical reactions, particularly the carbon compounds it assimilates. Data are based upon the complete reactions of 92 strains. A gaseous fermentation is produced in glucose. Galactose is seldom fermented, and the fermentation is weak when it does occur. Maltose, sucrose, lactose, and raffinose are not fermented. Esters are not produced. Carbon compounds assimilated by all strains are: glucose, galactose, L-sorbose, maltose, sucrose, cellobiose, trehalose, melezitose, soluble starch, xylose, L-arabinose, D-arabinose, D-ribose, rhamnose, D-glucosamine hydrochloride, ethyl alcohol, glycerol, mannitol, sorbitol, alpha-methylglucoside, salicin, pyruvate, succinate, and citrate. Compounds assimilated to a variable extent from strong to nil are: erythritol, dulcitol, potassium gluconate, calcium 2-ketogluconate, and ethyl acetoacetate. Not assimilated by any of the strains are the following compounds: lactose, melibiose, raffinose, inulin, potassium 5-ketogluconate, potassium sodium saccharate, lactate, and inositol. Nitrate is assimilated, starch is not synthesized, and an exogenous supply of vitamins is required for growth.

The sexually potent strain NRRL Y-2155 is designated as the type strain. Accessory strains are: Y-2154, a naturally occurring strain of sex opposite to Y-2155; (Y-2154 x Y-2155)-1, an inbred mating type which mates more abundantly with Y-2155 than does Y-2154; and (Y-2154 x Y-2155)-118, a bisexual diploid which sporulates slowly but abundantly. The type and accessory strains have been lyophilized especially for taxonomists and set aside for their use. It is intended that the lyophilized preparations will be opened and relyophilized at

15-year intervals. Since lyophilization should be the best means of reducing variation and supplying convenient live cultures containing both vegetative cells and ascospores, the lyophilized cultures maintained at this laboratory are designated as the type material.

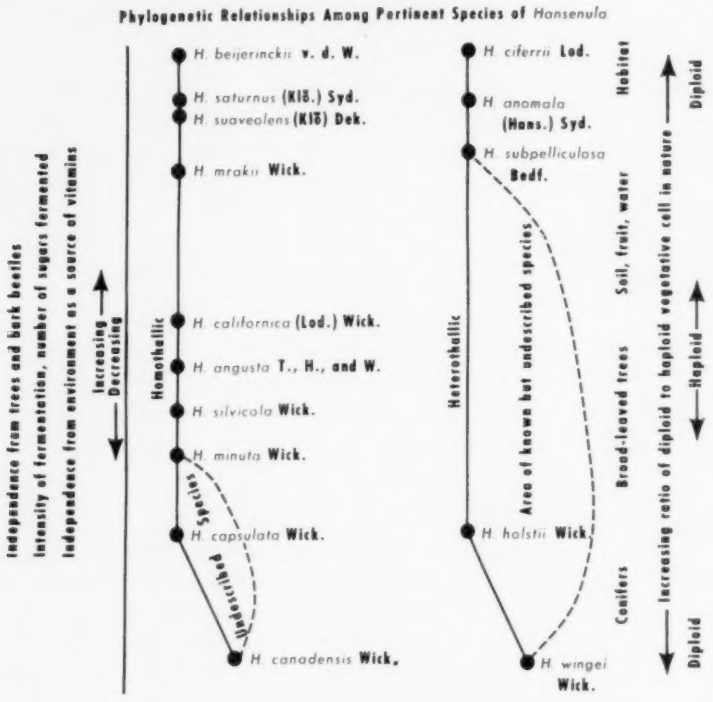

FIG. 1. Relationship of habitat, physiology, and ploidy in the evolution of some species of the genus *Hansenula*.

In 1947, Dr. Eugene C. Holst of the Division of Bee Culture, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Maryland, sent us cultures of *H. holstii* which he had isolated from the bark beetle *Dendroctonus engelmanni*. Dr. Holst's strains now are identified as a member of the genus *Hansenula;* we therefore take pleasure in naming the species in his honor. It was with deep regret we learned of his death in December 1954.

Hansenula holstii sp. nov.

Asci per conjugationem cellularum sexuum amborum orti. Ascosporae petasiformes numero ad 2 in quoque asco. Verum mycelium et pseudomycelium cum blastosporis. Coloniae nitidae at mucidae. Glucosum et galactosum (ad gradum varium) fermentatum, sed non maltosum, sucrosum, lactosum, nec raffinosum. Nitras, glucosum, galactosum, L-sorbosum, maltosum, sucrosum, cellobiosum, trehalosum, melezitosum, amylum, xylosum, L-arabinosum, D-arabinosum, D-ribosum, rhamnosum, glucosaminum, alcohole aethylicum, glycerolum, mannitolum, sorbitolum, alpha-methylglucosidum, salicinum, pyruvatum, succinatum, citratum, assimilata sed non lactosum, melibiosum, raffinosum, inulinum, potassium-5-ketogluconatum, sodium-potassium saccharatum, lactatum, nec inositolum. Assimilata ad gradum varium sunt erythritolum, dulcitolum gluconatum, calcium-2-ketogluconatum, acetoacetatum ethylicum. Isolata ex gummi *Pruni* et ex cuniculis coleopterarum larvalium sub cortice *Pini*.

DISCUSSION

Considerable difficulty was encountered in isolating sexually active strains of the heterothallic haploid species *H. holstii*. By backcrossing ascosporic isolates with their parents, mating types were obtained that react sexually to a far greater extent than the natural isolates from which the inbred types were derived. There is a tendency for even the most primitive species of *Hansenula* to produce diploid cells. In fact, the tendency is so extremely slight that this most primitive heterothallic species of the genus is not obtained from nature in the diploid form. A bisexual diploid was obtained in the laboratory. It sporulates slowly but abundantly after 3 or 4 weeks on a slant of sporulation medium. As much or more difficulty was encountered in obtaining ascosporogenous matings in the next heterothallic haploid species (to be described later) above *H. holstii*. Yet better sporulating, inbred mating types were obtained from it, as well as a sporogenous bisexual diploid.

Sometimes it is very difficult, other times easy, to find mating types for more or less primitive species in nature, but yeast taxonomists, we believe, are obligated to make the effort before naming a new species in the imperfect genera of *Candida* or *Torulopsis*. If the species exists in frass, the best possibility for obtaining sexually reactive strains, according to our experience, is from trees which are within a few hundred feet of one another. It is our intention that, when we find mating types of long-established species that have been described as nonascosporogenous, we shall continue the use of the species epithet if it is legitimate to do so. If the nonsporogenous species was described after 1952, the date of our first publication on the occurrence of heterothallic haploid yeasts in nature, we shall give it a different epithet. This procedure seems to be

in accordance with the spirit of the present International Rules, and may serve as a stimulus for taxonomists to search for mating types of any of their presumed new nonsporogenous yeasts which, because of their biochemical properties, may be anticipated to be haploid. The imperfect species corresponding to *H. holstii* is *Candida silvicola* Shifrine and Phaff (**1956**). This epithet could not be maintained for the species even if it were desired, for another species of *Hansenula* has already received the name *silvicola*.

SUMMARY

Hansenula holstii is judged to be the most primitive, or ancient, heterothallic species of the genus because it is so nearly exclusively haploid in the vegetative state that the diploid form has never been isolated from nature. This species ferments one sugar, rarely two, and the fermentation is slow. Even thin pellicles are seldom produced, and esters are not synthesized. *H. holstii* has the maximum requirement of the genus for vitamins. Its colonies are generally mucoid, and the cells are small. All of these characteristics of haploids contrast with the corresponding characteristics of those more recently evolved diploid yeasts which live independently of trees. *H. holstii* and *H. capsulata*, the latter being the most primitive homothallic species of the genus, live mainly in association with coniferous trees and bark beetles. From the taxonomic area in which these two species occur, a heterothallic and a homothallic line of species evolved into associations with deciduous trees and finally into free living forms. The physiological powers of these two lines increased with succeeding species. Two other lines, starting at the phylogenetic level of *H. holstii* and *H. capsulata*, developed into complete dependence upon coniferous trees, with concurrent gradual loss of the ability to cause gaseous fermentation. In all four lines the primary evolutionary characteristic was increase in the ratio of diploid to haploid vegetative cells. The proportion of diploid cells increased rapidly in the lines developing toward the free living state, but slowly in the lines developing toward dependence upon conifers. It is thus apparent that *H. holstii* and *H. capsulata* occupy important positions in the evolution of the genus.

Generally, the more primitive a heterothallic species of yeast is, the more difficult it is to find sexually reactive forms in nature, and *H. holstii* is no exception to this rule. However, by mating ascosporic isolates back to their parents through two successive experiments, inbred mating types were obtained which mated abundantly among themselves but not with natural isolates. Although diploid cells are formed in such

infinitesimally small numbers by primitive haploid species that diploid colonies are never isolated from nature, they may be produced in the laboratory, and pure ascosporogenous diploid cultures have been produced for the two primitive heterothallic species of *Hansenula* discovered to date.

Hansenula holstii produces in good yield an extracellular phosphomannan that has interesting and potentially useful properties.

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HANSENULA ANGUSTA, AN EXCELLENT SPECIES FOR DEMONSTRATION OF THE CO-EXISTENCE OF HAPLOID AND DIPLOID CELLS IN A HOMOTHALLIC YEAST

DOROTHEA J. TEUNISSON,[1] HARLOW H. HALL,[2] AND LYNFERD J. WICKERHAM[3]

Wickerham stated (**1951**) that the ratio of diploid to haploid cells is the dominant characteristic determining the evolutionary level attained by a species of *Hansenula*. This relationship holds whether the species is homothallic or heterothallic, and whether the yeast belongs to the phylogenetic lines which developed toward the free-living state or toward more dependence upon trees and insects. Other characteristics, such as increasing size of cells and transition from mucoid, to butyrous, to mat colonies, also reveal evolutionary succession in the various lines. Physiological characteristics, particularly the number of vitamins required from the environment, the number of sugars fermented, and the rate of fermentation, also indicate evolutionary position. The physiological reactions, however, became stronger in lines developing toward the free-living state, and weaker in the lines developing toward greater dependence upon insects and trees.

The genus *Hansenula*, as we now know it, apparently originated as a conifer-inhabiting species. The yeasts in the lines pointing toward independence from trees evolved successively as species dependent upon coniferous trees, then as species dependent upon deciduous trees, and finally as species living independently in soil and water. The completely dependent lines of *Hansenula* became limited in habitat to the coniferous trees. All lines leading to either dependence or independence showed an increasing ratio of diploid to haploid cells as succeeding species evolved,

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and many of the recently evolved species exist almost exclusively in nature as diploid cells.

HABITAT AND TAXONOMY

Although *Hansenula angusta* is relatively new and the name has not been validated previously by a Latin description, it is a species of wide distribution among certain deciduous trees. *H. angusta* was first studied, insofar as is known, by Teunisson and Hall (see Wickerham's description of the species, **1951**). It was one of *22* strains of osmophilic yeasts isolated from pasteurized, canned orange-juice concentrate (65° Brix, sugar content ca. 50%, pH 3.5) which had spoiled. It may have been carried to the oranges or juice by bark beetles or fruit flies, and the relatively high heat tolerance of its ascospores (10 minutes at 65° C) may have allowed the species to survive pasteurization. In thermal death rate studies on this strain in single-strength and concentrated orange juice, the cells survived heating at 85° for one minute and 80° for 5 minutes. It has been isolated a number of times since then from fruit flies (*Drosophila*) and their feeding places in mountainous areas of California by Shehata, Mrak, and Phaff (**1955**) and by Phaff, Miller, and Shifrine (**1956**). Wickerham has found *H. angusta* in compost of bagasse in Louisiana, and several times in the frass of species of deciduous trees, as follows:

Liquidamber styraciflua L. Redgum. Louisiana.
Nyssa sylvatica Marsh. Blackgum. Louisiana.
Populus tremuloides Michx. Quaking aspen. Minnesota.
Quercus alba L. White oak. South Carolina.
Quercus nigra L. Water oak. Louisiana.

H. angusta is rather common in frass of freshly fallen acorns which in the fall are hosts to larvae that feed upon the kernel. Incidentally, in freshly fallen, larva-infested acorns and hickory nuts there are frequently present interesting pink and white molds that produce a strong odor of esters which are evidently of high molecular weight.

The Latin description, prepared a few years ago by Dr. Ernst A. Bessey but not previously published, follows:

Hansenula angusta sp. nov.

Conjugatio heterogamica, at interdum fiunt asci ex transformatione cellularum vegetativarum diploidearum. In quoque asco formantur usque ad 4 petasiformes ascosporae. Limbus ascosporae angustus. Cellulae vegetativae spheroideae, 2.2–5.2 μ. Pseudomycelia et vera mycelia deficiunt. Coloniae sunt nitidae et butyraceae. Pelliculae super media liquida non formantur. Glucosum fermentatur at

non galactosum, maltosum, sucrosum, lactosum, nec raffinosum. Nitras, glucosum, maltosum, sucrosum, trehalosum, melezitosum, zylosum, D-ribosum, alcohole aethylicum, glycerolum, erythritolum, adonitolum, dulcitolum, mannitolum, sorbitolum, succinatum, citratum, assimilata, sed non lactosum, melibiosum, raffinosum, inulinum, amylum, glucosaminum, salicinum, calcium-2-ketogluconatum, K-5-gluconatum, Na-K-saccharatum, acetoacetatum ethylicum, nec inositolum. Galactosum, L-sorbosum, cellobiosum, L-arabinosum, D-arabinosum, rhamnosum, alpha-methylglucosidum, gluconatum, pyruvatum, lactatum, assimilata ad gradum varium. Vitamina externa necessaria.

The name *angusta* signifies the narrow brim of the approximately hemispheroidal ascospores. The strain selected as the TYPE is NRRL Y-2214, which was isolated from *Drosophila pseudoobscura* at Keen Camp, California, in May, 1950, by *Shehata* et al. (**1955**), *50–102*. Lyophilized cultures consisting of haploid and diploid, vegetative and sporulated cells are available at the Northern Regional Research Laboratory in Peoria, Illinois.

Hansenula angusta should prove to be an excellent example for classroom study of the occurrence of haploid and diploid cells in a yeast. It is among the lowest of those species considered to be of intermediate evolutionary development. General morphological characteristics which distinguish the two ploidy levels are adequately shown by this yeast. It sporulates readily on a vegetation medium commonly used for cultivation and isolation (yeast extract malt extract agar, or YM [4]), and the ascospores impart a red color to the colonies. Since diploid yeasts generally sporulate earlier and in greater abundance than haploid yeasts of the same phylogenetic line, the colonies stemming from diploid cells of *H. angusta* become pinkish at about four days, while the colonies of haploid cells are still white. As the culture ages, the diploid colonies become red, and the haploid colonies pink. The pigmentation starts in the area of confluent growth of the streak plate, and continues outward, but the differentiation among neighboring haploid and diploid colonies is clear. Occasionally it will be noted that the diploid colonies are slightly larger than the haploid colonies. It is generally true that the higher up the phylogenetic line a yeast is situated, the larger the diploid colonies will be in comparison to the haploid colonies. Since *H. angusta* is relatively low in its line, the difference in size of the colonies is slight, often not being discernible at all. In nature the ratio of diploid to haploid

[4] YM agar consists of 3 g each of yeast extract and malt extract, 5 g of peptone, 10 g of glucose, and 20 g of agar per liter. The pH is not adjusted. This medium is available from Difco. (Use of a company, or product, or both, named by the Department does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.)

cells of *H. angusta* is small, though so far as the authors are aware diploid cells nearly always accompany haploid cells, and the occurrence of white and reddish colonies on 4- to 6-day-old YM isolation plates strongly suggests that the isolate is *H. angusta*.

Microscopical observations of cells from the haploid colonies at 4 to 6 days may show up to 40 per cent ascospores, though few approach this maximum. The vegetative cells are small and possess more buds than the diploid cells, a comparison which holds generally for diploid and haploid cells of *Hansenula* and related genera. The asci stemming from haploid vegetative cells are conjugated, occasionally arising from two independent cells, but generally arising from mother and daughter cells. Usually the larger component contains the ascospores and the bud, having lost its nucleus, is an empty-appearing cell devoid of vacuoles, granules or other cytoplasmic structures, and its wall is thinner than that of the spore-containing component. The asci formed from haploid cells are smaller than asci formed from diploid cells.

Asci formed from diploid cells consist of a single cell. If the asci are produced on a rich medium, such as YM, the ascus is often associated with a daughter cell which arose from the mother cell as a bud before the mother cell sporulated. Thus it is common to see spores in one cell and attached to it a diploid bud which is large and has a very live appearance imparted by vacuoles and granules. Later, these large buds may sporulate too, and the colony may contain up to 80 or 90 per cent of spores. If the diploid culture is sporulated on a medium deficient in some nutrient, fewer of the asci are connected to an independent cell. Such a medium is malt extract sporulation medium (ME [5]) which is deficient in available carbohydrate.

Though the number of haploid cells of *H. angusta* evidently always greatly exceeds the diploid cells in nature, laboratory media generally tend to increase the percentage of diploid cells. The haploids show a constant, small tendency to diploidize while growing vegetatively, but the diploids evidently do not revert to the haploid form until sporulation occurs. A sporulated diploid culture may be streaked, and some haploid colonies will be produced from ascospores. By microscopic observations when the colonies are 6 to 10 days old, some of the haploid colonies may be found to contain only conjugated asci among the vegetative cells. Others will have both conjugated and unconjugated asci. Such colonies were derived from either a mixture of haploid and diploid cells, or from haploid cells some of which conjugated, the zygote nuclei then giving

[5] ME agar consists of 5 g of malt extract and 3 g of agar per 100 ml of water. The pH is not adjusted. It is prepared as slants.

rise to buds which reproduced asexually for a while before sporulating. The asci of the majority of the colonies may be exclusively unconjugated. The color of the colonies will, of course, increase in the order just given. Either ploidy level may be kept indefinitely in a practically pure state by lyophilization.

SUMMARY

Hansenula angusta is a species of yeast associated with deciduous trees and disseminated by bark beetles and fruit flies. It exists in nature predominantly in the haploid form but a small percentage of diploid cells are generally present. Ascospores impart a red color to the colonies when they are 4 to 8 days old, the diploid colonies having a denser color than the haploid colonies because diploid cells of homothallic yeasts generally sporulate sooner and more abundantly than the haploid cells. This species is recommended for classroom demonstration of the following general characteristics of homothallic yeasts which exist in both the haploid and diploid forms: Haploid cells are smaller than diploid. Haploid cells produce conjugation tubes whereas diploids do not, and the asci formed from haploids are smaller and are conjugated, while those produced by diploids are larger and are unconjugated. The haploid cells diploidize at a slow rate while growing vegetatively, but the diploids evidently must sporulate in order to return to the haploid state. Either form may be derived and maintained in practically pure state by selection and lyophilization.

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AN UNDESCRIBED SMUT DISEASE OF SOYBEANS [1]

MARVIN D. WHITEHEAD AND M. J. THIRUMALACHAR

(WITH 4 FIGURES)

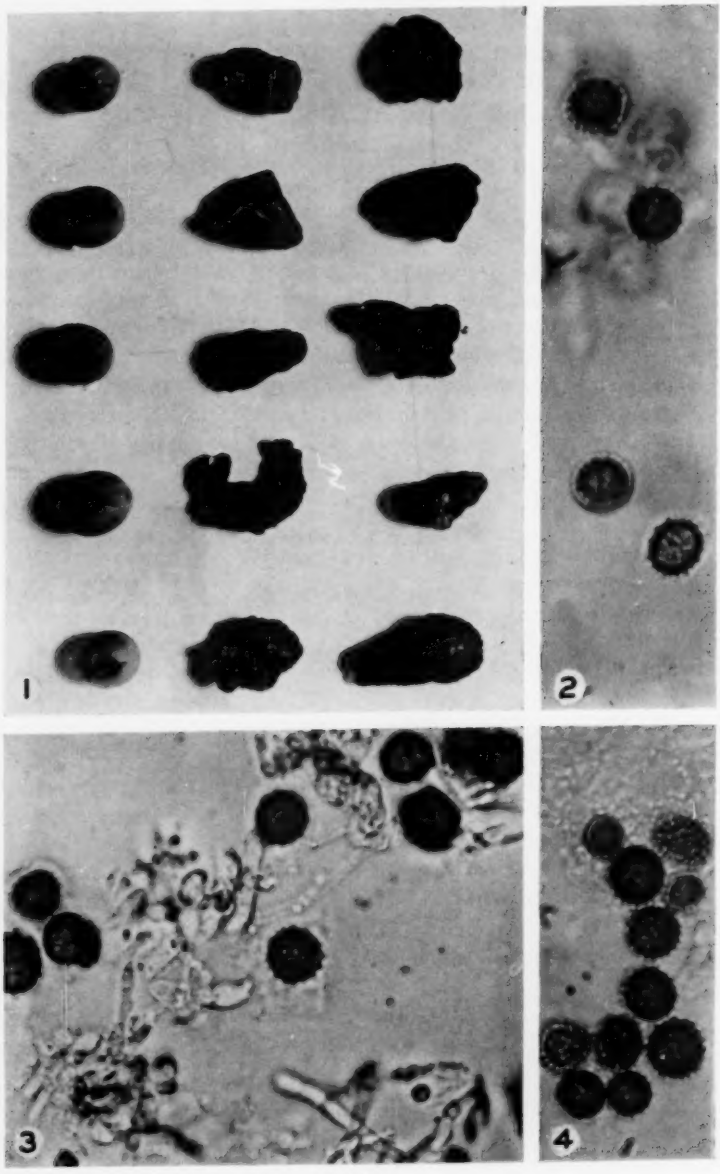
Galls of a smut were observed to occur in several samples of Missouri certified soybean seed grown during the 1954–1958 seasons. Nine lots of seed, including the varieties Dorman, Clark, Chief, and Ogden, grown in as many locations scattered throughout Missouri, were found contaminated. The galls were malformed soybean pods of hard, charcoal-like consistency, 0.3 to 0.9 cm in diameter and 0.5 to 1.5 cm long (FIG. 1). While in most cases the entire pod was transformed into a smut gall, in a few the infection was observed to be partial, with remnants of the soybean seed. One to three galls per thousand seeds were found in the seed lots.

Sections through the galls revealed the type of sorus typical of the genus *Melanopsichium* Beck. The sori were locular, showing 30–40 locules in a transverse section. The outer covering layers were hard and of the same cellular structure of a normal soybean pod. In the center of the gall, the seeds were replaced by a mass of thin-walled host cells in which the locules were differentiated, enclosing the black spore masses. The spore masses are nonpulverulent and held together in a gelatinous matrix.

Study of the sorus development indicated that the locules arose as lysigenous cavities. The hyphae bordering the locules were differentiated into numerous spores which were later pushed towards the center and held together in a gelatinous matrix. When stained with eosin B or congo red, both the young and old sori showed the gelatinous matrix embedding the spore mass. The mature spores were dispersed only after the disintegration of the sorus. There was no extrusion of the spore mass in the form of spore tendrils, etc., as seen in *Melanopsichium pennsylvanicum* Hirschh. (1). The spores are reddish brown,

[1] Acknowledgment is made to Andrew Tau and Edward D. Bishop for their assistance in the preparation of the photomicrographs and photograph; and to J. Ross Fleetwood and Viola M. Stanway for supplying certain samples.

Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 1972. Approved by the Director.



FIGS. 1–4.

subglobose to spherical, minutely and densely echinulate, measuring 6.1–11.3 × 7.5–12.0 μ with a mean of 8.7 × 9.8 μ (FIG. 2). The chlamydospores germinated readily when placed in water and incubated in a moist chamber at 24° C. The promycelium was septate, bearing sporidia both laterally and terminally. Numerous secondary sporidia were formed by budding.

Artificial cultures of the smut were prepared by aseptically transferring mature chlamydospores to petri plates of potato-dextrose agar. Following spore germination on potato-dextrose agar, a mycelial type of colony developed, which was at first white and crusty and later turned hard and greyish black. Microscopic examination revealed that the mycelium became closely septate and rounded off into chlamydospores following gelatinization of cell walls (FIG. 3). Unlike many other smuts, the chlamydospores were not merely thick-walled irregularly formed cells, but closely resembled in size, shape, spore echinulations, etc., the naturally formed ones in the host (FIG. 4). In general, the type of growth in artificial culture resembled that of *Ustilago neglecta* Niessl which forms predominantly chlamydospores in artificial culture.

The hard charcoal type of sorus enclosing the spore masses in locules of host tissue indicates close relationship with the genera *Melanopsichium* and *Pericladium* Pass. (*Xylosorium* Zundel). Even though the latter genus is based partly on the type of chlamydospore germination (single terminal sporidia), the chief characteristic feature is the absence of the gelatinous matrix embedding the spores within the sorus. The genus *Melanopsichium* is characterized by the presence of a gelatinous matrix in the sorus in addition to its locular nature. The smut under study is an undescribed species of *Melanopsichium* for which the name *M. missouriense* sp. nov. is proposed.

Melanopsichium missouriense sp. nov.[2]

Gallas duras brunneo-atras non erumpentes, 0.3–0.9 cm latas 0.5–1.4 cm longas per transformationem leguminum efformans; sori loculares, 200–300 μ in diametro, cellulis matricis compositi, in sectione transversali 30–40 loculis visis; chlamydo-

[2] The authors are indebted to Edith K. Cash, Mycologist, Division of Mycology and Disease Survey, U.S.D.A., for the preparation of the Latin diagnosis.

Melanopsichium missouriense. FIG. 1. Smut galls compared with seeds of Clark variety soybean seeds. FIG. 2. Photomicrograph of spores from smut galls (× 627). FIG. 3. Photomicrograph of spores and mycelium formed in culture showing rounding-off process of spore formation (× 627). FIG. 4. Photomicrograph of chlamydospores formed in culture showing characteristic development of echinulations (× 627).

sporae e mycelio loculos circumdante centripetaliter natae, rufo-brunneae, in massa atrae, in matricem gelatinosam immersae, non pulverulentae, subglobosae usque ad sphaericales, minute denseque echinulatae, 6.1–11.3 × 7.5–12.0 μ (medianae 8.7 × 9.8 μ), in maturitate e promycelio septato sporidia terminalia et lateralia ferente germinantes.

Inciting the formation of hard galls by the transformation of pods, brownish black, nonerumpent, 0.3 to 0.9 cm broad, 0.5 to 1.4 cm long; sori locular, 200–300 μ in diameter, 30–40 locules in cross section, composed of host cells. Chlamydospores formed from mycelium bordering locules centripetally, reddish brown, black in mass, embedded in gelatinous matrix, nonpulverulent, subglobose to spherical, minutely and densely echinulate, measuring 6.1–11.3 × 7.5–12.0 μ (mean 8.7 × 9.8 μ). Mature spores germinating by septate promycelium bearing terminal and lateral sporidia.

HABITAT: Parasitic on pods and seed of *Glycine max* (L.) Merrill collected at Macon (TYPE), Poplar Bluff and Mexico, Missouri, September, 1954; Oregon, Trenton and New Madrid, Missouri, September, 1955; Jefferson City, Missouri, September, 1956; Brunswick, Missouri, September, 1957; and Fayette, Missouri, September, 1958.

Portions of the type specimen collected at Macon, Missouri, are deposited in the mycological herbarium of National Fungus Collection, Beltsville, Maryland; New York Botanical Garden, New York, New York; Harvard University, Cambridge, Massachusetts; University of Wisconsin, Madison, Wisconsin; and University of Missouri, Columbia, Missouri.

SUMMARY

A new species of smut found infecting soybeans is described as *Melanopsichium missouriense*. The soybean pod is consumed, and a gall of hard, charcoal-like consistency is formed. Chlamydospores, resembling as to spore form, echinulations, etc., those formed in the galls, develop readily in culture. Chlamydospores germinate forming promycelia bearing sporidia both laterally and terminally.

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THE MORPHOLOGY OF LEPTODISCUS TERRESTRIS, AND THE FUNCTION OF SETAE IN SPORE DISPERSAL [1]

DONALD V. MCVEY AND J. W. GERDEMANN

(WITH 16 FIGURES)

Leptodiscus terrestris Gerd. is a common parasite of several forage legumes (**5**). The fruiting body of this fungus was originally described as a superficial, radiate stroma one cell layer thick, and it was considered to most nearly resemble an acervulus (**4**).

A more detailed study has been made on the fruiting structure in an effort to determine its affinity, and the origin and development of conidia have been more thoroughly investigated. The conidia of *L. terrestris* have a relatively long filamentous seta at each end. A study was made to determine the position of the setae relative to the spores when the spores are contained in the spore head, and to determine if the setae function in spore dispersal.

Setulate conidia are produced by many fungi; however, there are few reports in which a function has been attributed to them. Atanasoff (**2**) found that the bristles borne on the conidia of *Dilophospora alopecuri* (Fr.) Fr. became attached to the body of a nematode and that the nematode transported the spores to the growing point of cereals. Cunnell (**3**) found that the setae of *Robillarda phragmitis* were folded back against the side of the spore and that they became extended when the spore was released. He suggested that the appendages might function as a flotation device.

Length of time required for sporulation. Leaf spots develop on alfalfa, *Medicago sativa* L., when leaflets are artificially inoculated with spores of *L. terrestris*. However, fruiting does not occur on these spots unless the leaflets are removed and placed in a moist chamber. The length of time required for spore formation was determined by removing infected leaflets from the plant, washing them in 1% Clorox (5.25% sodium hypochlorite), and placing them on moistened sterile filter paper

[1] Portion of a thesis submitted by the senior author to the Graduate College of the University of Illinois in partial fulfillment of the requirement for the degree of Doctor of Philosophy. This study was supported by the North Central Forage Crops Technical Committee NC-37.

in Petri plates. The plates were incubated in diffused light and the leaflets were examined periodically under a stereoscopic microscope. Fruiting structures were removed and mounted in lactophenol for detailed examination.

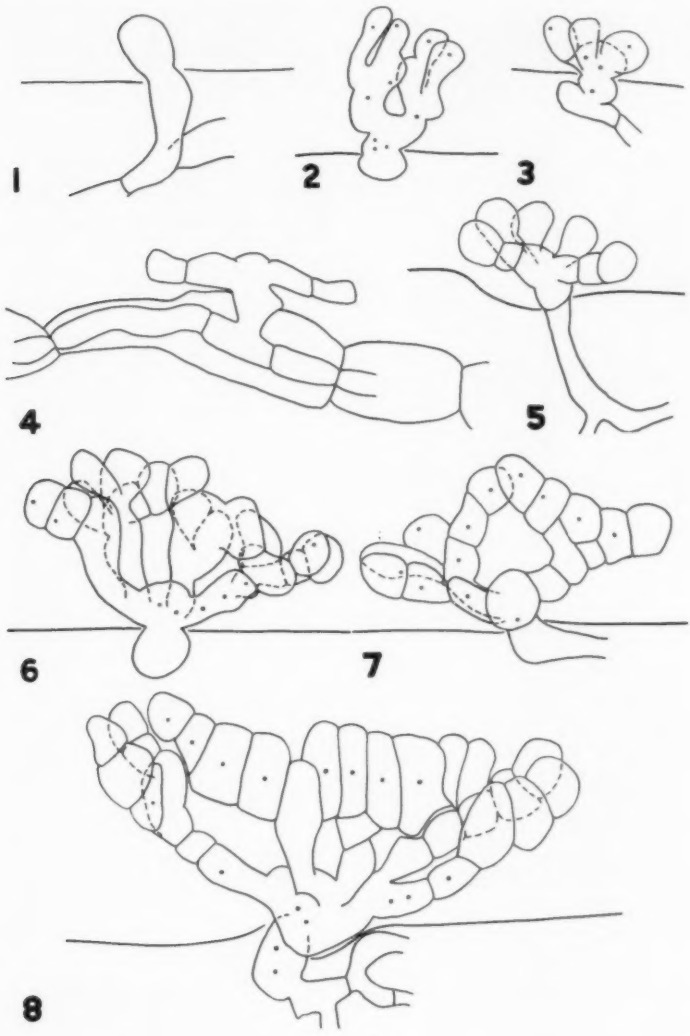
Fruiting structures began forming on the evening of the third day after the infected leaves were placed in a moist chamber, and continued to develop throughout the night. Spore formation began the following morning about 4 AM, and the first spores were mature between 7:30–8:00 AM. Spore formation continued throughout the day as new fruiting structures formed; however, it appeared that only one crop of spores was produced each day from a fruiting structure.

Effect of light on formation of the fruiting structure and on sporulation. L. terrestris was cultured on propylene-oxide-sterilized bluegrass leaves floated on 2% water agar in Petri plates. Five plates were incubated in the dark in controlled temperature chambers at 5, 10, 15, 25, and 30° C. At the same time, 5 plates were placed on a laboratory table in daylight. After 5 days fruiting structures and spores were present in the plates exposed to light. After 2 weeks no fruiting structures or spores were found in the plates incubated in the dark at any temperature.

The fungus was also cultured on propylene-oxide-sterilized alfalfa stems floated on water agar. Five plates were placed on a laboratory table in the light, and on the same laboratory table 3 groups of 5 plates each were incubated in the dark in separate containers. Plates incubated in the dark were removed at 5-day intervals, and the stems were examined for fruiting structures. Plates incubated in the light were examined after 5 days. Fruiting structures and spores occurred only on cultures kept in the light.

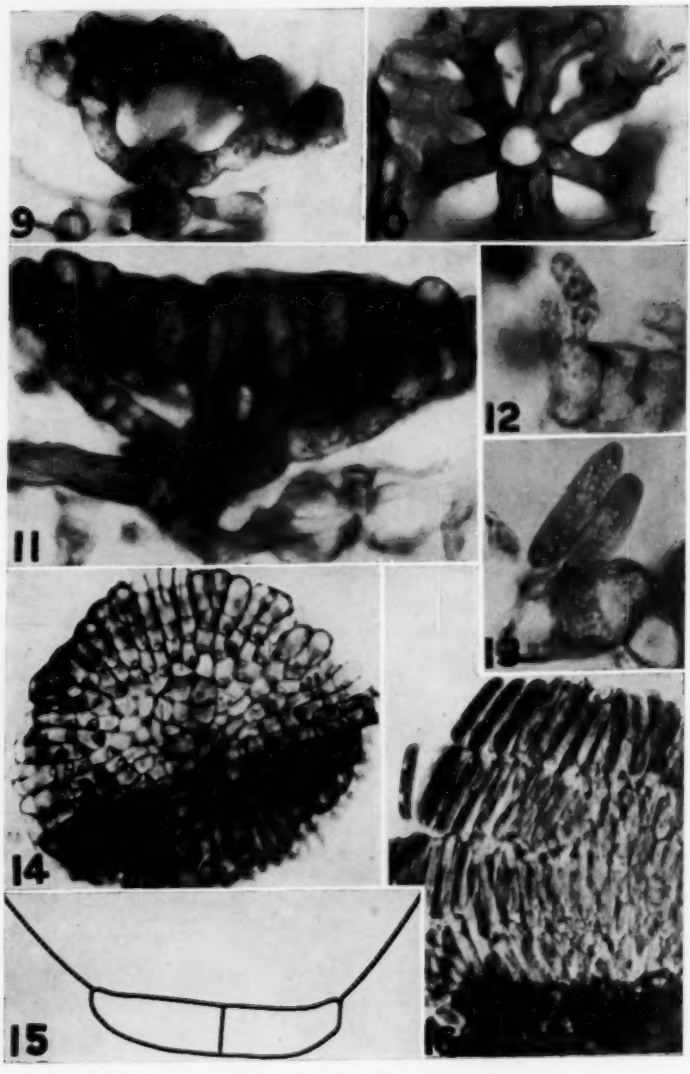
In another experiment cultures containing immature fruiting structures without spores were placed in containers and incubated in the dark for as long as 3 days. Sporulation did not occur as long as the cultures remained in the dark. Upon exposure to light, mature spores were observed within 3½ to 4 hours. Therefore, it appears that light is necessary for initiation of both the fruiting structure and the spores.

Developmental morphology of the fruiting structure. L. terrestris was studied using infected leaflets from artificially inoculated alfalfa. Leaflets were surface sterilized and incubated on moist sterile filter paper in Petri plates. Developing fruiting structures and spores were examined as whole mounts in lactophenol and as stained paraffin sections. When leaflets were first removed from plants the hyphae within lesions were intercellular and averaged 2.7 μ in diameter. Forty-eight hours later the hyphae growing from lesions were still predominately inter-



Figs. 1–8.

Figs. 1–8. Stages in the development of the fruiting structure of *Leptodiscus terrestris*, × 990. 1. The initial cell formed from a hypha within an epidermal cell of a leaflet. 2, 3. Vertical branches arising from the initial cell. 4. Horizontal branches arising from the initial cell. 5. Enlarged cells at the apex of branches. 6, 7. Early stages in the formation of sporogenous cells. 8. Mature fruiting structure.



FIGS. 9–16.

FIGS. 9–16. Stages in the development of the fruiting structure and spores of *Leptodiscus terrestris*. 9. Initial cell, and branches with immature sporogenous cells at their tips, × 1030. 10. Upper surface of a young fruiting structure showing

cellular and were of 2 distinct sizes averaging 2.7 μ and 5.4 μ in diameter, respectively. After 72 hours, the hyphae were both intercellular and intracellular and hyphae averaging 5.4 μ in diameter predominated; however, hyphae as much as 10 μ were common.

The fruiting structure is meristogenous, developing from a single cell (Fig. 1). This cell usually arises from a hypha in an epidermal cell of the leaflet (Fig. 4); however, it occasionally develops from a hypha in the mesophyll. The first cell formed is usually hourglass-shaped (Figs. 1, 9). The lower portion of the initial cell develops within an epidermal cell and the upper portion projects slightly above its surface. Septate hyphae grow from the upper portion of the initial cell (Figs. 2–5, 10). These hyphae branch and grow vertically (Fig. 2) or somewhat horizontally (Figs. 4, 10), and are slightly constricted at each septum (Figs. 7–9). The apex of each branch enlarges (Fig. 5) and produces a sporogenous cell (Figs. 6, 7, 9). At maturity, sporogenous cells are slightly rounded on the top and bottom and the sides are irregular to nearly straight (Figs. 8, 11). Each newly developed sporogenous cell enlarges and produces another cell. When the space between 2 radial rows of sporogenous cells becomes great enough, a cell divides to form another radiating row of cells that fills the space. At maturity the fruiting structure is a saucer-shaped radiating disk one cell-layer thick (Fig. 14). Branches from the initial cell often pass under the disk until they reach the edge, where they produce more sporogenous cells (Fig. 8), causing the fruiting surface to appear to have several points of origin.

Dark staining of hyphae near the fruiting structure indicates a concentration of protoplasm at this location. The initial cell is multinucleate (Figs. 8, 9) with 2, 3, or 4 nuclei in the lower portion and as many as 9 in the upper portion. Cells of the branches are predominately uninucleate although a few contain 2 nuclei. The sporogenous cells are uninucleate with the nucleus usually located near the center of the cell (Fig. 8.).

The macroscopic appearance of the fruiting structure of *L. terrestris* suggests that it is either an acervulus or a sporodochium; however, a more detailed examination clearly shows that it cannot be classed as

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branches radiating from the initial cell, × 1030. 11. Initial cell, branches, and sporogenous cells of a mature fruiting structure, × 1030. 12, 13. Immature spores attached to sporogenous cells by short stalks, × 1030. 14. Upper surface of a mature fruiting structure showing the beginning stage of spore development, × 450. 15. A mature spore showing the slightly flattened lower end at the right, × 1900. 16. Mature spores in a spore head, × 450.

either structure as they are conventionally defined (1). The possibility is suggested that it may be a large, complex, highly developed conidiophore.

Spore formation, attachment and release. Spores develop on the upper surface of the fruiting structure. Spore formation begins as a small protuberance near the inner edge of each sporogenous cell (FIG. 14). These protuberances enlarge and elongate and as they develop, they appear to be attached to the sporogenous cell by a very short stalk (FIGS. 12, 13). When the spores are mature, this stalk is no longer visible. The spores are borne vertically with the concave surface toward the center of the fruiting surface. The upper end of the spore forms a continuous, smooth curve and the lower end tends to be slightly flattened at the point where the spore was attached to the sporogenous cell. Each sporogenous cell of a fruiting structure produces a spore at the same time and successive crops of spores are produced to form a spore head in which the spores are arranged in distinct layers (FIG. 16). The spores adhere tightly together, indicating that they are embedded in a mucilaginous substance.

Setae develop after the spores reach their mature size. The seta on the upper end of the spore is produced at the point of greatest curvature toward the concave side, and the seta on the lower end develops at the edge of the slightly flattened surface on the concave side of the spore (FIG. 15). One septum regularly develops at or near the middle of the spore. However, a few spores have been observed with 2 and 3 septa.

The release of spores was observed by placing partially dried spore heads on a glass slide and covering them with a cover slip. The slide was placed under the microscope and the spore head located. Water or lactophenol was slowly added at one edge of the cover slip. When water came in contact with the spores, they dispersed in all directions so rapidly that it was impossible to determine what had occurred. However, when lactophenol came in contact with the spores, dispersion was slower, and it was possible to determine that in the spore head the setae are folded back against the spore wall on the concave side. As the setae unfold, the spores at the edge of the spore head are pushed outward with considerable force. At the top of the spore head the setae on the upper ends of the spores push the spores away from each other, and the setae on the lower end of the spores force the spores upward and out of the spore head. As this action occurs, the peripheral layer of spores is forced outward from the spore head. As soon as the peripheral layer begins to move, the next layer commences and so on until all the spores

are released. When this action takes place rapidly, the spore head appears virtually to explode as spores are forced outward in all directions.

Menispora tortuosa Corda, a fungus having setulate conidia similar to those of *Leptodiscus terrestris,* was studied in an attempt to determine the mechanism of spore release. The spores of *M. tortuosa* are produced from phialides and they form in small slimy fascicles.

Fascicles of spores carefully mounted in lactophenol remain intact, and as long as the spores adhere together setae are not visible. If the setae were to extend outward from the tips of the spores, as they do when the spores are free, they should project outward from the ends of the fascicle. It therefore appears that, when the spores are bound together in mucus, the setae are folded back against the sides of the spores.

Fascicles of spores were placed on a dry slide and covered with a cover slip. The fascicles were located under the microscope and water was slowly added at one edge of the cover slip. When water came in contact with the fascicles, the individual spores broke away with a quick jerky motion similar to that observed with spores of *L. terrestris.* After the spores were free, the extended setae were visible. The setae of *M. tortuosa* are much finer and hence more difficult to observe than those of *L. terrestris,* and it was not possible to determine their position before spore release or to actually see setae unfold. However, the available evidence strongly suggests that they are folded back along the concave side of the spore and that they become extended when the mucus is dissolved in water. The occurrence of the same mechanism of spore dispersal in two apparently unrelated fungi suggests that this phenomenon may be of common occurrence among fungi having setulate conidia.

SUMMARY

Leptodiscus terrestris requires light for the formation of both fruiting structures and conidia. The fruiting structure is meristogenous in origin. Branches are produced from an initial, multinucleate, hourglass-shaped cell. Sporogenous cells develop at the apex of each branch, and these in turn produce more sporogenous cells, eventually forming a thin, saucer-shaped fruiting structure.

The spores first appear as small protuberances on the inner edge of the sporogenous cells. These protuberances enlarge and elongate and as they develop, they appear to be attached to the sporogenous cells by a very short stalk. This stalk disappears when the spores mature. When the spores reach their mature size, the setae are formed. The

seta on the upper end of the spore develops at the point of greatest curvature toward the concave side, and the seta on the lower end develops at the edge of the slightly flattened surface near the concav side of the spore. One crop of spores is produced by the sporogenous cells each day. Spores are held together in a head by a mucilagenous substance.

When the spores are in the head, the setae are folded back against the spore wall on the concave side. When the mucus is dissolved, the setae unfold and expell the spores outward with considerable force. The same mechanism of spore dispersal also appears to occur in *Menispora tortuosa*, a fungus with setulate conidia similar to those of *L. terrestris*.

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THE SUBMERGED CULTURE OF MORCHELLA [1]

FRANK A. GILBERT

INTRODUCTION

Lambert (**9**) some years ago successfully grew the mycelium of *Agaricus campestris* in submerged culture, but Humfield (**5**) appears to have been the first to suggest a potential economic future in mycelial production of edible mushrooms. Following Humfield's lead, a number of investigators started work on the problem and it was soon found that several species and strains of species were adaptable to this method of cultivation.

Agaricus bisporus and its progenitor *A. campestris,* because of their familiarity and general acceptance, were among the first to be tried. Humfield (**6**) isolated and tested more than forty strains of *A. campestris* and found three particularly well adapted to submerged culture. Block and his associates (**1**), investigating the problem of the disposal of citrus press water in Florida, studied its use as a medium for the submerged growth of *Agaricus blazei,* a southern species which can be grown at a higher temperature than *A. bisporus.* Subsequently, Block, Humfield, and others studied the submerged cultural characteristics of many other species of edible fungi. A summary of such findings has been published by Eddy (**3**).

From an experimental point of view, and so far as production has been concerned, research on the submerged culture of *A. campestris* and other agarics has been most successful but, because the full flavor of the pileus does not occur in the mycelium, future commercial exploitation of agaric mycelium seems dim. To quote Block (**1**), "Fundamental questions that remain to be answered are whether the true mushroom flavor is a product of only the specialized cells of the fruiting body and cannot be produced by the mycelium, or whether precursors normally found in soil or manure but not in the laboratory media are required for synthesis of the flavoring compounds in the mycelium."

Meanwhile Joseph Szuecs (**14**), a pioneer in submerged culture techniques, and the holder of several patents in this connection, had also

[1] This work was supported in part by a grant from the Producers Creamery Company.

screened many species of mushrooms and other edible fungi for their ability to grow in submerged culture. He reduced the number of adaptable species to relatively few including a particularly desirable *Morchella*. Szuecs had collected this *Morchella* in an orchard at Yonkers, New York, in April 1947. A successful tissue culture was made but, unfortunately, the sporophore was not preserved for accurate identification. Szuecs was later provided with a culture of *Morchella hortensis* Boud. by Professor Paul Haudoroy of Lausanne, Switzerland, and, as the cultural characteristics of the American and European specimens appeared to be similar, Szuecs considered his *Morchella* to be *M. hortensis* even though this species had not been described from North America. The species or strain, whatever its identity, proved to be very adaptable to submerged culture. It had many desirable characteristics and appeared to be outstanding in its potential for commercial production. Moreover, the full flavor of the sporocarp was carried in the mycelium. Szuecs after several years work in studying nutritional characteristics and improving cultural techniques, chiefly on a laboratory scale, was granted a patent (**13**) that gave him broad protection on the commercial growth of *Morchella* and other members of the Helvellaceae in aerated liquid media. A subsequent patent (**15**) extended this coverage to include the submerged aerobic growth of the mycelium of all mushrooms used for food.

The Szuecs strain of *Morchella* has been engineered to experimental commercial-scale production (**10**) but no previous study has been made in which the characteristics of different species of *Morchella* in submerged culture have been compared.

MATERIALS AND METHODS

The ease with which *Morchella* mycelium can be grown has been known for years. The sporocarps are easy to collect, and cultures are not difficult to obtain, either from tissue transfers or from the ascospores. The omnivorous character of the mycelium allows it to be grown submerged on an extremely wide range of nutrient materials. Typical suitable carbohydrates are hydrolyzed starch, maltose, glucose, xylose, sucrose, lactose, carboxymethylcellulose and the like, and also alcohols such as mannitol and glycerol.

The nitrogen may be supplied by ammonium salts, nitrates, urea, or amino acids; or by proteins such as albumin, peptone, cottonseed meal, peanut meal, coconut meal, soybean meal, and wheat bran. Although *Morchella* is capable of synthesizing tissue with 20 per cent to 45 per cent protein from a purely synthetic solution where the only organic component is glucose and the only nitrogen source is ammonium phosphate,

corn steep liquor has been used as an additional ingredient in experimental commercial production and in most laboratory and pilot plant runs. A trace element mixture is always added as a precautionary measure. It appears quite possible that any future commercial production of the mycelium will make use of available waste products.

Morchella is propagated on agar slants in the usual manner, both in the laboratory and in experimental commercial production. In starting a "run," the contents of as many slants as needed are homogenized in a sterile "blendor" and the finely divided mycelium used to "seed" a number of shake flasks. These are agitated for approximately 72 hours on a rotary shaker set at a speed of about 100 r.p.m. The mycelium agglomerates into a large number of small nodules from one to several millimeters in diameter. With a lower rate of agitation the mycelium tends to grow together to form a few large aggregates, and at a higher speed the mycelium will not nodulate well.

At the laboratory scale, 500 ml Erlenmeyer shake flasks each containing 100 ml of inoculated nutrient are used. The amount of inoculum in one flask serves to further inoculate seven liters of nutrient in a ten-liter aerated culture bottle or carboy. In experimental commercial production, six-liter shake flasks, each containing approximately two liters of nutrient, are used, and the contents of several flasks are used to inoculate a tank. The exact number of flasks depends on the size of the tank. Unlike the case in yeast production, a portion of the finished product cannot serve as inoculum for the next batch. Also, unlike the procedure in antibiotic production, sparged air alone is used for both aeration and agitation. An impeller is not used since violent agitation would tend to prevent the formation of discrete spheres of mycelium so desirable in *Morchella* mycelium production.

With the growth of the mycelial nodules the medium clears in approximately 48 hours and the mycelium may be harvested as early as 72 hours. On a laboratory scale, however, it has been found that an additional 24 hours in the bottles gives a smoothness or "polish" to the mycelial spheres, which vary in size from one-fourth to one inch in diameter. This maturity allows the moisture to be extracted from the spheres at harvest with less felting of the mycelium. Felting is a very undesirable feature if the product is to be used fresh or is to be frozen.

SPECIES

As has been stated, Szuecs spent several years, before obtaining his patents, in establishing nutritional and cultural requirements for his strain of *Morchella*. He grew many species of fungi in submerged

culture, and probably other Morchellas, but published no comparisons of cultural differences between species or strains of species. Much of our information on the submerged culture of *Morchella* in the laboratory, and all of our knowledge of large scale production, has been obtained with what we have called the Szuecs strain.

In the past two years nine additional species, and numerous strains of several of these species, have been grown in submerged culture. Some of these were collected by the writer, some were given or sent to him for identification, and some were received on agar slants from several sources both in this country and in Europe. Most were from tissue cultures but some were grown from single ascospores.

Boudier (**2**), in his work on the "morels" of France, recognized 23 species not all of which appear to be distinct. Lagarde (**8**) points out the close relationship between some of Boudier's species. The "morel" known in this country as *Morchella esculenta* is largely *Morchella rotunda* of Boudier but, to judge from the numerous published illustrations of American specimens, may be one of several Boudier species.

Seaver (**11**), in his monograph of the North American operculate discomycetes recognized six North American species, all of which have been recorded from Europe. Although treating *M. conica* as a species, he questioned its validity. Groves and Hoare (**4**) would further reduce the North American species of *Morchella* to three. They also questioned the validity of *M. conica* and, in addition, believed that *M. deliciosa,* represented an immature stage, and *M. crassipes* an over-mature stage, of the polymorphic *M. esculenta.* We have grown in submerged culture all six of the North American species with the exception of *M. deliciosa,* a specimen or culture of which we have not yet been able to obtain. To judge from the cultural characteristics, there appears to be no doubt that *M. crassipes* is quite distinct from *M. esculenta.*

RESULTS

CULTURAL DIFFERENCES. Variations occur in cultures of *Morchella,* not only between species but between strains of the same species. For example, three strains of *M. angusticeps* obtained from ascospores from the same collection of ascocarps have shown recognizable differences both on agar and in submerged culture. On the other hand, *M. esculenta, M. conica,* and *M. vulgaris* have certain similar cultural characteristics, presumably indicating a fairly close relationship.

In submerged culture, using a standard nutrient formula, the following differences are distinguishable: rate of growth, habit of growth, development of discrete mycelial spheres, color of spheres, flavor of mycelium,

intensity of flavor, color of the supernatant liquor, and odor of the effluent air.

The Szuecs strain, which by comparison of cultural characteristics indeed appears to be *M. hortensis* or closely related to it, has not been surpassed so far as the rate of growth is concerned. This strain and those of *M. hortensis* will reach cultural maturity, in the standard media used, at least 24 hours sooner than any other species tried.

Some species and strains will form firm discrete mycelial spheres in submerged culture, either from freshly homogenized mycelium or from mycelial pellets developed in a shake flask. Other strains never form firm spheres, whether or not they form pellets in shake flasks. Some strains form soft spheres which later fragment into irregular particles. Such mycelium felts badly while being centrifuged or squeezed, and makes an inferior product for some culinary uses. The spheres vary in size from one-fourth to one inch in diameter and average about one-half inch, but tend to be larger in some strains.

M. crassipes, in culture, develops the largest, firmest, most resilient spheres of all. These never felt together, even under pressure sufficient to reduce the mycelium to less than a 75 per cent water content. The Szuecs strain, *M. hortensis, M. rimosipes,* and some strains of *M. esculenta* also form discrete spheres but show a greater tendency to felt under pressure than *M. crassipes.* The spheres of some of these strains and species also show an undesirable tendency to coalesce if the agitation caused by aeration is insufficient. On the other hand, too violent an agitation causes fragmentation.

The color of the spheres is not an absolute criterion of species differentiation and is somewhat variable, possibly depending on the degree of caramelization of the nutrient sugar when being autoclaved. The variation is from white to dark gray. However, the fresh spheres of *M. rimosipes,* strains of *M. hortensis,* and the Szuecs strain are nearly always white, those of *M. crassipes* light gray, and those of strains of *M. esculenta* darker gray. The mycelial cake of strains not forming spheres has the same color variations. The color of the spheres tends to parallel the color of the supernatant liquor, the whitest spheres coming from species or strains in which the liquor becomes honey or straw colored and the darker spheres from strains in which the liquor is amber to chestnut. The odor of the effluent air tends to be uniform within species and to differ between species. For example, the odor of the effluent air from *M. rimosipes, M. hortensis,* and the Szuecs strain is always quite aromatic or "estery," that of *M. esculenta* somewhat earthy, and that of *M. angusticeps* reminiscent of "slippery elm." The odor of

the air from most of the others may be described as "farinaceous." Color, however, and to some extent the odor can be altered by changing the medium. Lactose, for example, when used as a carbohydrate source always produces a dark mycelial product and a very dark supernatant liquor.

In taste panel tests, the flavor of the mycelium of *M. crassipes* was deemed the most delicious and was preferred to all others without exception. That of *M. esculenta* was second, and that of *M. hortensis* and the Szuecs strain, third and fourth. The tastes of *M. angusticeps, M. conica, M. vulgaris,* and *M. rimosipes* were acceptable but not as well liked as those of the first four. The taste of two strains of *M. semilibera* was quite bland.

TABLE 1

CULTURAL CHARACTERISTICS OF SPECIES OF MORCHELLA

Species	Development of mycelial spheres	Flavor	Rate of growth
Strains of *Morchella crassipes* (Vent.) Pers.	Excellent	Excellent	Good
Szuecs strain	Good	Good	Excellent
Strains of *Morchella hortensis* Boud.	Good	Good	Excellent
Strains of *Morchella esculenta* (L.) Pers.	Good to Fair	Excellent to Good	Good to Fair
Morchella rotunda Boud. (strain of *M. esculenta*)	Good	Good	Good
Morchella vulgaris (Pers.) Boud.	Fair	Fair	Fair
Strains of *Morchella conica* Pers.	Fair to Poor	Fair	Fair
Strains of *Morchella angusticeps* Peck	Poor	Fair	Fair
Morchella rimosipes D.C. (strain of *M. semilibera*)	Good	Fair	Poor
Strains of *Morchella semilibera* D.C.	Good to Fair	Bland	Fair to Poor

Intensity of flavor was determined by defrosting the frozen mycelium, squeezing it, and noting the persistence of the odor. In regard to intensity of flavor, the Szuecs strain was not surpassed.

The amount of mycelial growth can be changed by altering the nutrient medium. Although *Morchella* will grow quite well in many media, it does not produce the maximum amount of mycelium in all. Flavor appears to be a genetically controlled character in *Morchella* and the characteristic flavor of each species has been obtained in cultures of that species regardless of the medium. Unlike *Tuber* and many species of agarics, *Morchella* develops its characteristic flavor in the mycelium as well as in the sporocarp.

A comparison of the cultural characteristics of several *Morchella* species is shown in TABLE 1.

TEMPERATURE.—*Morchella* can grow at lower temperatures than most fungi. In the writer's laboratory several flasks often are run simultaneously on the shaker. The flasks that are not used immediately for inoculation are placed in the cold room at a temperature of 36° F.

Even at this temperature, growth takes place and if the flasks are not used in a few days, they must be discarded because the pellets tend to grow together.

Carboys were inoculated and placed in refrigerators set at five degree intervals from 40° to 60° F. At 40° F growth was slow and the mycelial spheres were harvested at ten days instead of the usual three or four days. As the five degree temperature intervals went up, the rate of growth increased up to 55° F. This was the lowest temperature at which the spheres could be harvested 72 hours after inoculation of the carboy. The optimum range appears to be from 55° to 70° F. Although *Morchella* has been grown when the laboratory temperature was more than 80°, the maximum growth temperature has not been established. An advantage of growing *Morchella* at the lowest temperature commensurate with good growth is the comparative freedom from contamination at this temperature. The temperature experiment was carried out with the Szuecs strain. Absolute ranges of temperature for growth may not be identical in different species.

PRODUCTION. Sugihara and Humfield (**12**) obtained 20–25 g dry weight per liter of *Morchella crassipes* and *Morchella* sp. mycelium grown in submerged culture. Eddy (**3**) obtained 23.6 g dry weight per liter of *Morchella esculenta* mycelium grown in the same manner.

In our laboratory and in experimental commercial runs, production has been somewhat less. We obtain per liter about 100 g of mycelial pellets with approximately a 90 per cent water content. In one experimental commercial run, 3200 lbs of drained but not squeezed mycelium was obtained from 1600 gallons of media. The water content was not determined.

One advantage that *Morchella* has over many other edible fungi in submerged culture is the lack of production of secondary spores similar to those described by Kligman (**7**). Where such spores are formed, a heterogeneous mass of mycelium is produced which is not very acceptable if the product is to be frozen or used fresh.

The process using both a shake flask and a final stage as described earlier in this paper is desirable from a commercial standpoint because the two stages may be run simultaneously. The "seeding" of the large tanks may be made with small mycelial nodules rather than with newly homogenized mycelium and the time of the run shortened by at least one day. It has been found, however, that in the laboratory it is quite possible to inoculate a carboy with homogenized mycelium directly and in four to seven days, depending on the species or strain, to achieve the same results.

ACCEPTANCE AS FOOD. Because of the difficulty in obtaining the desirable flavor of the pileus in agaric mycelium, some have doubted whether the flavor in the *Morchella* sporocarp and in the mycelium is identical. A comparison was made between frozen "morels" from Michigan (mostly *M. esculenta*) and the frozen mycelium of the Szuecs strain. A comparison was also made between fresh *M. esculenta* sporocarp and freshly cultured *M. esculenta* mycelium. The products were fried in butter in the same manner and submitted to a taste panel. The panel found the flavors similar but not identical. There was no preference for the sporocarp over the mycelial product. In other tests the panel showed preference for the mycelium of some species over that of others but all were recognizable as *Morchella*.

The mycelium has been used fresh, fresh-frozen, dried, powdered, and prepared as a flavor concentrate. *M. crassipes, M. hortensis,* some strains of *M. esculenta,* and the Szuecs strain are desirable as a fresh or a fresh-frozen product because the whitish or grayish compressed spheres have a very pleasing appearance in addition to their superior flavor. Any species may be dried, or dried and powdered. The powdered mycelium has been kept for more than two years without loss of flavor when used in soup. It has been found possible to prepare a flavor concentrate by freezing the mycelium and concentrating the extract from the defrosted product. The concentrate from this process has kept more than a year under refrigeration and at the present writing has not lost its strength.

Dr. James Y. P. Chen, Professor of Pharmacology at Marquette University School of Medicine, made a thorough investigation of the toxicity of *Morchella* mycelium grown in submerged culture. Extrapolating from experiments with rats he found that a person would have to consume 60 pounds of the mycelium per day before there would be any ill effects. The product has been approved by the Pure Food and Drug Administration.

SUMMARY

Nine species of *Morchella,* and a number of strains of several of these species, have been grown in submerged culture.

Morchella in submerged culture is quite omnivorous so far as its carbohydrate and nitrogen requirements are concerned.

The mycelium develops the piquant flavor of the sporocarp.

In submerged culture, using a standard nutrient formula, the following species and strain differences are distinguishable: rate of growth,

habit of growth, development of discrete mycelial spheres, color of spheres, flavor of mycelium, intensity of flavor, color of the supernatant liquor, and odor of the effluent air.

The mycelium will grow at temperatures as low as 36° F, but growth is much slower than at 55 to 70° F, a range which appears to be optimum.

A production of 10–25 g (dry wt.), of mycelium per liter of nutrient medium may be expected.

The mycelium may be utilized fresh, fresh-frozen, dry, powdered, or as a flavor concentrate.

At least one strain has been shown to be adaptable to commercial production.

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YEASTS IN POLLUTED WATER AND SEWAGE

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INTRODUCTION

The presence of cells or colonies of yeasts in polluted water, sewage, and sewage-treatment processes may have been anticipated by some workers dealing with the biology of such habitats, but little has been said about them in the literature. Cake yeast has been used in the starting of septic tanks, activated-sludge systems, and sludge digesters, but whether brewer's or baker's yeast was more effective or effective at all is unknown.

Heukelekian (**1942**) set up a series of experiments with cake yeast, using a brand of baker's yeast supplied by Anheuser Busch Co., adding it to sewage in open and closed vessels, and to a ripe sludge mixture. He concluded that "(1) yeast does not hasten the stabilization of sewage under anaerobic conditions," and "(2) yeast does not hasten the digestion of seeded sludge mixtures." In 1953 Heukelekian again reported on an experiment in which baker's yeast was used to determine the effect of this type of biological preparation on the digestion of sewage solids. He concluded that "the addition of . . . yeast does not increase the liquefaction of non-sterile fresh solids as measured by the B.O.D. of the supernatant liquor," although during the experiment some gas was formed and solids were reduced to some extent.

That no recovery was made of either baker's or brewer's yeast from samples tested for fungi, in the series of isolations on which this report is based, may result from an error in sampling technique, from incomplete sampling due to use of incorrect enrichment medium, or from a habitat not conducive to the survival of these species of *Saccharomyces*.

In screening various polluted habitats for their potential fungus populations, in most of them yeasts were found to be present, sometimes in large numbers. A collection of about 275 cultures of these organisms has been accumulated and the following is a report on those species which occur in part of this collection.

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ISOLATION TECHNIQUES

The primary isolation techniques for fungi described earlier (Cooke, **1954**) always yielded some colonies of yeasts and, where differences appeared significant, these colonies were picked and held for study. L. J. Wickerham has suggested in correspondence an additional technique for isolating yeasts.

A solution of yeast nitrogen base (YNB, Difco) was prepared with 1% glucose and another with 20% glucose. These were dispensed at the rate of 50 ml to each of the required number of 250-ml Erlenmeyer flasks. To a pair of flasks, one of each sugar concentration, was added 2 ml of a 1:10 dilution suspension of the sample to be tested. The flasks were then shaken on a rotary shaker for 60–72 hours, removed, and the contents allowed to settle. Yeast cells settled to the bottom, bacteria, if present, remained suspended longer, and fungus mycelium (in chunks or balls) floated or remained suspended. At the end of 4 hours the flask could be tilted, and the yeast sediment picked up on a loop and streaked on a plate of yeast-extract-malt-extract-glucose agar. After three days or less the growth was transferred to slants or to additional dilution plates for sorting into the separate species which developed. Because of the use of enrichment cultures, no claim is made that all possible yeasts were obtained or that the yeasts which were obtained were the more important in the tested sample.

IDENTIFICATION TECHNIQUES

Identification of cultures was carried on in the laboratories of the Department of Food Technology, University of California at Davis. The techniques used were those of the Dutch workers, described by Lodder and Kreger-van Rij (**1952**), and of Wickerham (**1951**). The medium in which assimilation of carbon sources was tested was Wickerham's (**1951**) yeast nitrogen base as prepared by Difco. Yeast carbon base was used for testing the utilization of nitrogen sources. The auxanographic technique was used for both carbon- and nitrogen-assimilation experiments. Inoculum was grown on heavily inoculated wort-agar slants. For all wort-agar, liquid-wort or malt-agar preparations, Fleischmann's diamalt was used in a concentration of 10° Balling. Streak cultures were prepared on wort-agar slants. Ring and pellicle formation was observed on liquid wort as was thallus formation and cell size. Utilization of ethanol as a carbon source was observed in liquid yeast nitrogen base to which 4% ethanol was added before autoclaving and which is approximately a 3% solution after autoclaving. The ability of

a culture to grow on a vitamin-free medium was studied in liquid culture using Wickerham's vitamin-free yeast base (Difco). The development of pseudomycelium was studied in slide cultures. Potato-dextrose agar, prepared in the laboratory, was poured on a glass slide in a Petri dish. The slide was placed on a bent piece of glass tubing and a small amount of sterile water was added to the plate to reduce the rate of drying. The agar was inoculated with two streaks, using a flat needle, and a cover glass was placed over part of the streaks. Aerobic and anaerobic types of growth could be observed beside and under the cover glass. Sporulation was tested on various agars—wort, potato-dextrose, vegetable, Gorodkowa's, or YM.

In the following discussion of the distribution of the isolated yeasts in sewage and polluted water, the extent of variation of each species or variety will be noted in relation to the "standard descriptions" given in the cited yeast manual. Minor differences in cell size are not considered important because of differences in techniques, types of cells measured, number of strains investigated, etc. Streak cultures will not be compared because of the extreme variability found within certain species both in our cultures and in reports in the literature (see, e.g., Lodder and Kreger-van Rij, **1952**). However, certain physiological and morphological characters which are relatively constant within a species will be considered.

HABITATS SAMPLED

Samples of various types have been taken from three kinds of polluted streams and from three types of sewage-treatment plants.

At Lytle Creek, Clinton Co., Ohio, eight stations were sampled in clean, recovery, and polluted zones. At each station water, water sediment in pools and in riffles, and saturated bank soil subject to overflow during floods were sampled. Water was sampled in several streams in southeastern Ohio, which carried acid mine wastes.

At Lawrenceburg, Indiana, a small stream carrying untreated wastes from a pharmaceutical plant to Tanner's Creek was sampled several times, especially in reference to slime growth on the creek bank and on debris in the creek on which slimes developed.

All the habitats sampled at the Dayton, Ohio, sewage-treatment plant yielded yeasts, but while some species appeared in a number of locations in the plant, insufficient data are available to develop distribution patterns. TABLE I lists the several types of locations which were sampled. The columns headed "habitat type" represent an effort to describe in one plane the three-dimensional flow chart of this plant. Several brief

notes may help clarify the flow pattern of sewage liquids and solids through the plant.

Grit (rags, wood, snails and other materials) is removed from raw sewage and discarded. The smaller solid materials then settle out. This material, now called sludge, is drawn off to digestion tanks. The scum which forms on the settling basin (or earlier on the primary tanks which perform a similar function) is drawn off and incinerated. Following settling, the liquid is drawn off and is passed through trickling filters,

TABLE I

OCCURRENCE OF SPECIES OF YEASTS IN STAGES OF THE DAYTON, OHIO, SEWAGE-TREATMENT PLANT

Stage	Habitat type			
	Liquid system	Solid system	Permanent population	Discard materials
Raw sewage	6			
Detritus				3
Pre-aerated sewage	2			
Settled sewage	6			
Scum, surface of primary settling (or Imhoff) tank				2
Slime, high-rate trickling filter			3	
Slime, low-rate trickling filter			3	
Filtered sewage	9			
Final sewage	6			
Primary settled sludge		7		
Secondary digested sludge		1		
Digested sludge freshly poured on drying beds		3		
Medium-dry sludge		3		
Air-dry sludge		6		
Heat-dried sludge		2		
Vacuum-filter cake				1

Numerals indicate numbers of species isolated at stages in habitat types.

the biology of which has been described elsewhere (Cooke and Hirsch, **1958**; Cooke, **1958**, **1959**). The filtered sewage picks up sloughed materials as it passes through the filters. These are settled out in the final settling basin before the liquid is discharged to the Great Miami river. The final settling-basin sludges are returned to detritus tanks in which snails, sewage earthworms, and other larger materials which developed in the treatment plant are removed and discarded.

The sludge in the primary settling tanks is drawn off into digestion tanks where bacteria (and possibly some fungi) act upon it, reducing its organic content. During retention in some of the tanks, the sludge is

heated to 95°–98° F by burning gases derived from the digestion process. Following digestion, one of two processes is used to dewater the sludge which now contains between 3.5 and 5.0% solid matter. Sludge may be poured on sand beds where the moisture is drained off below or evaporated from above, or it may be dried in a vacuum filter. Depending on the season, and whether the sand bed is under glass or in the open, the sludge dries to 60% or more solid matter in 1 to 4 months. When the sludge reaches about 60% or more dry matter it is removed from the drying beds, ground, and passed through chambers heated to 300–400° F in a multihearth furnace. It is then pulverized and sacked for sale as an organic soil conditioner. About half this material is mineral matter; the remainder is incompletely-decomposed cellulose and humus materials. When sludge is dried in a vacuum filter the cake is incinerated.

The small activated-sludge treatment plant at Yellow Spring, Ohio, has been sampled. The in-plant locations which were studied include plant influent, sewage in the early- and late-stage aerator tanks, sewage in the final settling tank, growth on the wall of the final settling tank, plant effluent, sludge in the bottom of the digester, medium-dry and air-dry sludge on the drying beds, and water in the creek carrying the effluent of the plant to Glen Creek at 200 ft and ¼ mile from the outfall.

The Ithaca, New York, sewage-treatment plant was sampled once by M. A. Rosinski. This is a primary-type plant without biological treatment. Sampling locations included (1) the influent channel, (2) the effluent channel, (3) a short distance away from the plant in the effluent, and (4) a mile out in Cayuga Lake.

At the Glendale, Ohio, sewage-treatment plant a series of air samples was obtained in the spring of 1953 by M. J. Foter and R. S. Lloyd by attaching calibrated liquid impingers, sieve samplers, and aerosol and hydrosol membrane filters to a manifold. Known volumes of air were passed through these samplers located within a few feet of a chamber into which recirculated sewage and raw sewage influent were poured continuously. The resulting spray was tested to determine the microbial population of the air and the efficiency of the samplers. Air samples were cultured for bacteria, yeasts, and molds.

The Pullman, Washington, and Moscow, Idaho, sewage-treatment plants were surveyed for fungal populations during the autumn, winter, and spring seasons, 1952–1953 (Becker and Shaw, **1955**). Among the isolates from this study which were sent to Cincinnati for identification or confirmation for which exact habitat information is not available were several yeasts.

At the Department of Public Health Experiment Station, Lawrence, Massachusetts, Albert E. Feldman sampled slimes on a number of experimental trickling filters for fungal populations. Cultures of the molds and yeasts he found were forwarded to Cincinnati for identification.

YEAST SPECIES ISOLATED

SACCHAROMYCETACEAE

Hansenula anomala (Hansen) H. & P. Sydow

Dayton, Ohio. Isolated once from the effluent of a low-rate trickling filter. April 14, 1953. (SEC 2101).

SEC 2101 is a strain which causes fermentation and utilization of galactose and develops a thin pellicle on liquid wort.

Hansenula mrakii Wickerham

Isolated once from bank soil at Station 5.2 in the upper part of the lower recovery zone on Lytle Creek, Clinton County, Ohio. June 12, 1952. (SEC 1295).

This species is found frequently in nature in other habitats such as slime fluxes of trees (Phaff and Knapp, **1956**). Neither this strain nor a number of others could be induced to produce ascospores on a variety of media at Davis or by Wickerham at Peoria. However, since it fits Wickerham's description of *H. mrakii* in all other points, it is assigned to this species rather than described as a new imperfect species.

Saccharomyces drosophilarum Shehata, Mrak, & Phaff

Lytle Creek, Clinton Co., Ohio. Two strains were isolated from stream water at station 8.7. This station was above the principal source of pollution and what wastes it carried came from scattered dwellings and farms above the city of Wilmington. June 12, 1952. (SEC 1297, 1298).

Previously this species has been isolated from slime flux of *Quercus kelloggii* in California (Phaff and Knapp, **1956**), and from various wild species of *Drosophila* and *Aulacigaster*. (Shehata et al., **1955**; Phaff et al., **1956**).

The two isolates obtained in Ohio check well with the description of the species from California published by Shehata et al. (**1955**).

Saccharomyces heterogenicus Osterwalder

Dayton, Ohio. Isolated once from settled sludge in the bottom of Imhoff tanks. Sept. 25, 1952. (SEC 1515).

While ascospores could not be found in this culture on a variety of media, it is assigned here because it corresponds in every other way with cultures of this species.

Schwanniomyces alluvius Phaff, Miller & W. B. Cooke, Antonie van Leeuwenhoek **26**: 183. 1960.

Isolated on two separate occasions in the zone of recovery (station 5.0) and just below this zone (station 2.8) and below the outfall of the effluent from the Wilmington sewage-treatment plant on Lytle Creek, Clinton Co., Ohio. Both times it was isolated from stream-bank soil which was always saturated with stream water and always subject to flooding. July 10 and Sept. 18, 1952. (SEC 1310, 1466).

This new species will be described in a separate publication. The two known species of this genus have both been isolated from soils (Capriotti, **1957**).

CRYPTOCOCCACEAE

Candida curvata (Diddens & Lodder) Lodder & Kreger-van Rij

Dayton, Ohio. Isolated from a late stage in the preaeration tank. Nov. 5, 1956. (SEC 2930).

SEC 2930 agrees with the standard description. It does not grow in a vitamin-free medium.

Candida guilliermondii (Cast.) Langeron & Guerra

Dayton, Ohio. One strain isolated from raw sewage in the effluent from the detritus tank. Jan. 30, 1953. (SEC 1801).

Yellow Springs, Ohio. One strain isolated from air-dry sludge, Oct. 9, 1952 (SEC 1561), and one from water in the creek carrying the effluent from the treatment plant about ¼ mile below the outfall, July 31, 1952. (SEC 1280).

Lytle Creek, Ohio. Three strains isolated respectively from creek water at station 7.6, 0.4 mile above the outfall of the Wilmington sewage-treatment plant, from water and sediment in a riffle at station 4.2 in the zone of recovery, and from saturated bank soil at station 1.0 in the lower clean-water zone. June 12, 1952. (SEC 1290, 1292, 1294). SEC 1290 and 1292 were isolated with the use of a membrane filter using media and dilutions described earlier (Cooke, **1954**).

Data available on the six cultures assigned to this species indicate a fairly good conformity to the standard description. Lodder and Kreger-

van Rij indicate that galactose fermentation is negative or weak; in our cultures one was negative, one weak, and 4 were latent. In the last, galactose fermentation proceeded slowly, yielding ½ to ¾ vial of gas at the end of 14, 17, or 20 days. There was no fermentation of maltose. Melibiose was not fermented by strains SEC 1292, 1280, and 1290; No. 1294 produced only bubbles in its fermentation; only half a vial of gas was produced by 1561 in 20 days, but 1801 fermented this sugar fairly rapidly, filling a shell vial in six days. Strain 1801 assimilated sucrose weakly. All six strains grew well in the presence of ethanol as a sole carbon source.

While two strains appear to fall more nearly into *C. melibiosi* Lodder & Kreger-van Rij, according to Wickerham and Burton (**1954**) there is no basis for maintaining this as a distinct species.

Candida humicola (Daszewska) Diddens & Lodder

Dayton, Ohio. Four strains isolated respectively from raw sewage in the effluent from the detritus tank, from filtered sewage in the effluents from both high-rate and low-rate trickling filters, and from air-dried sludge. Oct. 30, 1952, Mar. 28, 1955, Nov. 5, 1956, and Feb. 11, 1958. (SEC 1651, 2941, 3047, 3096).

Ithaca, New York. One strain isolated from raw domestic sewage in the influent to the sewage treatment plant. Sept. 2, 1952. (SEC 1394).

Of the five cultures assigned to this species all conform well to the standard description. No. 3047 produced a thin pellicle and a fragile ring on liquid wort in contrast to the very coarse, rugose, granular pellicles produced by the other cultures. Ethanol as a sole carbon source was well utilized in all cases.

Candida intermedia (Ciferri & Ashford) Langeron & Guerra

Dayton, Ohio. One isolate came from settled sewage in the primary-settling-basin effluent, and another from sludge settled out in the same basin. Nov. 11, 1956, Feb. 2, 1958. (SEC 2934, 3071).

Yellow Springs, Ohio. Three strains were isolated, respectively, from the influent to the sewage-treatment plant, from medium air-dry sludge, and from water in the creek carrying the outfall from the plant about 200 feet downstream. May 29, 1952. (SEC 488, 1273, 1275).

The five cultures assigned to this species fit the standard description well in most points. However, all grew well when ethanol was supplied as the sole carbon source; Nos. 1273 and 1275 did not form a pellicle on liquid wort.

Candida krusei (Cast.) Berkhout

Dayton, Ohio. Four cultures isolated respectively from slime samples scraped from the stone of a high-rate trickling filter at the one-foot level, from the effluent of a low-rate trickling filter, from digested sludge freshly poured on the drying beds, and from medium-dry sludge. June 19 and July 17, 1952, April 14, 1953. (SEC 1283, 1285, 1303, 2100).

Yellow Springs, Ohio. Three strains isolated from scrapings from the wall of the final settling tank, from the treatment-plant effluent, and from digested sludge at the bottom of the digester. May 29, 1952. (SEC 487, 1274, 1277).

The seven isolates assigned to this species agree well in most characters with the standard description. Greatest amount of variation occurs in characters of surface growth on liquid wort. The formation of a pellicle varies from a thin white film to a thick wrinkled growth.

Candida parapsilosis (Ashford) Langeron & Talice

Dayton, Ohio. Isolated from raw sewage effluent from the detritus tank, Imhoff-tank scum, filtered sewage in the effluent from both high- and low-rate trickling filters, final sewage in the final settling basin, and freshly poured sludge on the drying beds. June 19, July 17 and Sept. 25, 1952, Apr. 14, 1953, and Apr. 1, 1954. (SEC 1301, 1308, 1515, 2104, 2105, 2109, 2117 and 3010).

Glendale, Ohio. Isolated on a hydrosol membrane filter sampler from air near the receiving tank of the sewage-treatment plant, and on a sieve sampler at the same point, Feb. 5 and Mar. 17, 1953. (SEC 1853, 1961).

Sunday Creek, Ohio. Isolated from a water sample taken one mile north of Shawnee, Perry Co., in a creek carrying acid mine wastes. April 10, 1954. (SEC 2166).

Most of the eleven isolates assigned to this species agree well with the standard description of the species. In some strains galactose was fermented moderately well, others produced a vial of gas in 6 to 12 days. Ethanol was used as a sole source of carbon by all strains. In SEC 1853 it is of interest that pseudomycelium formation is extremely reduced.

Candida pulcherrima (Lindner) Windisch

Dayton, Ohio. Isolated from detritus removed from settled sewage. Nov. 5, 1956. (SEC 2960).

The one strain assigned to this species ferments galactose but requires adaptation to it since gas was not produced until after 7 days

incubation and the vial was only ¾ full after 15 days. This strain formed very little pseudomycelium on potato-dextrose-agar slide cultures.

Candida rugosa (Anderson) Diddens & Lodder

Dayton, Ohio. One isolate was obtained from detritus removed from raw sewage which included recirculated sludge from the final settling tank. Single isolates were also obtained from sewage-treatment-plant effluent and from primary settled sludge from the Imhoff tanks. Feb. 11, 1958 (SEC 3060), June 19 and Oct. 30, 1952. (SEC 1304, 1661).

Another isolate was obtained from an experimental trickling filter in Lawrence, Mass., in 1955 (SEC 2349). SEC 2349 and 3060 fermented glucose weakly in contrast to the standard description of this species. Strains 2349, 3060, and 1661 utilized alcohol well. SEC 2349 did not produce a pellicle on liquid wort.

Candida tropicalis (Cast.) Berkhout

Dayton, Ohio. Nine isolates were obtained from the following sources: settled sewage in the primary-settling-basin effluent, filtered sewage in the low-rate-trickling-filter effluent, slime on the trickling-filter stone in the high-rate trickling filter at the one foot level, settled sludge at the bottom of the Imhoff tanks and the primary settling basin, digested sludge from the bottom of the secondary digester, and air-dried sludge. June 19, July 17, Oct. 30, 1952, Nov. 22, 1954, Nov. 5, 1956, and Feb. 11, 1958. (SEC 1281, 1300, 1302, 1307, 1660, 2242, 2933, 2937, 3088).

Yellow Springs, Ohio. One culture isolated from digested sludge. May 29, 1952. (SEC 1274).

Ten strains from among the isolates studied have been assigned to this species. Ethanol was used as a sole carbon source by all ten strains. No. 3088 formed neither a ring nor a pellicle in malt broth. Various types of pellicle were formed by the other nine strains ranging from islets to thin or thick smooth pellicles. It should be noted that No. 1281 appears to be intermediate between *C. tropicalis* and its variety *lambica*. Further tests are required to determine the extent of this relationship.

Candida utilis (Henneberg) Lodder & Kreger-van Rij

Dayton, Ohio. One culture isolated from filtered sewage in the effluent from a low-rate trickling filter. Nov. 22, 1954. (SEC 2234).

This isolate conformed well with the standard description of the species. It utilized nitrite as a sole source of nitrogen and grew fairly well in the absence of vitamins.

Cryptococcus albidus (Saito) Skinner

Glendale, Ohio. One strain isolated through a capillary impinger from air around the influent well at the sewage-treatment plant. Jan. 29, 1953. (SEC 1856).

The one strain assigned to this species agrees with the standard description except that the cell size in wort was larger than indicated for Group II cells and the cells were oval rather than globose.

Cryptococcus laurentii (Kufferath) Skinner

Dayton, Ohio. One strain was isolated from sludge which had dried in the air on sand and brick drying beds, and another from similar sludge which had been ground up, passed on a conveyor through a chamber heated to 200–300° F and pulverized. April 20, 1956. (SEC 2860B, 3053).

Glendale, Ohio. One strain was isolated with a sieve sampler from air near the influent well at the sewage-treatment plant. Feb. 5, 1953. (SEC 1854).

All three cultures assigned to this species were able to assimilate galactose well, but with ethanol as a sole carbon source, No. 1854 could not grow while 2860B and 3053 grew well in it. In liquid wort none of the cultures formed a pellicle.

Rhodotorula glutinis (Fres.) Harrison

Dayton, Ohio. Five cultures were isolated from the following sources: settled sewage as it entered the trickling filters, filtered sewage in effluent from a high-rate trickling filter, slime scraped from high-rate-filter stone, medium-dry sludge, and from air-dried sludge. Apr. 3, June 19, Aug. 14, Oct. 30, 1952, Apr. 14, 1953. (SEC 287, 1306, 1315, 1663, 2106).

Yellow Springs, Ohio. Three strains were isolated from activated sludge in the final aerator tank, from water in the effluent stream 200 feet from the plant, and from air-dry sludge. May 29, Oct. 9, 1952. (SEC 1276, 1279, 1560).

Glendale, Ohio. Four strains were isolated from air around the influent well at the sewage-treatment plant through the Schipe impinger and the sieve sampler. Feb. 10, 12, March 4, 10, 1953. (SEC 1888, 1889, 1958, 1959).

Lytle Creek, Ohio. Four strains were isolated from water and sediment in pools at station 2.8 at the lower end of the lower zone of recovery, and at station 8.7 in the upper clean-water zone, from saturated bank

soil at station 6.5 and station 7.2 in the zone of pollution. June 12, Sept. 18, 1952, Jan. 14, 1953. (SEC 1296, 1438, 1497, 1726).

Snow Creek, Ohio. One strain isolated from creek water at Murray City, Hocking Co., in a stream carrying acid mine wastes. April 10, 1954. (SEC 2166A).

Within the limits of the species as interpreted by Lodder and Kreger-van Rij (**1952**), 17 cultures of *Rhodotorula glutinis* have been isolated. Those with small cells (8 cultures) were placed in the variety *rubescens*. Four of these strains had a weak assimilation of galactose, two of sucrose, and the same two, Nos. 2106 and 1889, of maltose. Only one of the 17 strains, No. 1958, did not grow in the presence of ethanol as a sole source of carbon.

Rhodotorula graminis di Menna

Glendale, Ohio. One strain was isolated from air around the influent well at the sewage-treatment plant through the sieve sampler. Feb. 5, 1953. (SEC 1855). This yeast was described by di Menna (1958), who found it to be the dominant species on pasture grasses in New Zealand.

Identification was based on comparison with the type strain.

Rhodotorula minuta (Saito) Harrison

Dayton, Ohio. One strain was isolated from settled sludge from the bottom of the Imhoff tank. Sept. 25, 1952. (SEC 1511).

Yellow Springs, Ohio. Another strain was isolated from digested sludge from the bottom of a digester. Oct. 9, 1952. (SEC 1559).

Both cultures assigned to this species had characters similar to those given in the standard description. Slight variations were noted in that No. 1559 assimilated galactose weakly, and No. 1511 produced islets on the surface of liquid wort.

Rhodotorula mucilaginosa (Jörg.) Harrison

Dayton, Ohio. Five strains were isolated from raw sewage in the effluent from the detritus tank, final sewage in the treatment-plant effluent, settled sewage in the Imhoff tanks, air-dry sludge on the drying beds, and heat-dried sludge. Oct. 30, 1952, Jan. 30, Feb. 12, and April 14, 1953. (SEC 1659, 1802, 1887, 2103, 2107).

Lytle Creek, Ohio. Three cultures were isolated, respectively, from water at station 7.6 in the upper recovery zone, from saturated bank soil

at stations 6.5 in the polluted zone, and from 7.6 in the upper recovery zone. Mar. 5, Sept. 18, 1952. (SEC 275, 1475, 1493).

Acid streams, Ohio. Two strains were isolated from water in streams carrying acid coal-mine wastes: Sunday Creek, Corning, Perry Co., and Snow Creek, Murray City, Hocking Co. April 10, 1954. (SEC 2144, 2154).

Pullman, Washington-Moscow, Idaho. During development of a project (Becker and Shaw, **1955**) based on the fungi of the sewage-treatment plants of these two communities in 1952–3, Becker sent a strain of this species (SEC 2182) to Cincinnati for further study. *Rhodotorula* isolates, including this strain, were found in all effluent habitats sampled in both plants.

The seven strains of *Rhodotorula mucilaginosa* gave reactions comparable to the standard description of the species. Deviations occurred in certain areas. Six of the cultures utilized galactose more strongly than the others or than is indicated for the species, all were able to use ethanol as a sole source of carbon, and on liquid wort all degrees of pellicle formation were observed from no pellicle at all to islet formation, development of an incomplete film, a very thin or thin film, and in one instance to formation of a well-developed pellicle.

Rhodotorula rubra (Demme) Lodder

Lytle Creek, Ohio. One culture was isolated from saturated bank soil at station 7.6 in the upper recovery zone. Sept. 19, 1952. (SEC 1493).

The culture assigned to this species does not differ essentially from the standard description.

Rhodotorula texensis Phaff, Mrak & Williams

Sunday Creek, Ohio. One culture was isolated from water in a stream carrying acid coal-mine wastes, below Corning, Perry Co. April 10, 1954. (SEC 2144).

It is of interest that this species was first isolated from surfaces of shrimp from the Gulf of Mexico, a salt-water habitat (Phaff et al., **1952**).

Torulopsis candida (Saito) Lodder

Dayton, Ohio. Four strains were isolated from raw sewage in effluent from the detritus tank, final sewage in a final settling tank, slime scrapings from surface stone in a low-rate trickling filter, and freshly

poured sludge on the drying beds. April 14, 1953, March 28, 1955, Feb. 11, 1958. (SEC 2098, 3029, 3039, 3098).

Yellow Springs, Ohio. One culture was isolated from treatment-plant influent. May 29, 1952. (SEC 489).

The five cultures assigned to this species agree fairly well with the standard description. No. 3029 was able to ferment glucose weakly. Gas was not formed until the 8th day of incubation and a full vial of gas was obtained after 12 days. None of the cultures fermented sucrose and all grew well on lactose. No. 3029 did not produce a pellicle on liquid wort.

Torulopsis famata (Harrison) Lodder & Kreger-van Rij

Dayton, Ohio. Five strains were isolated from raw sewage in detritus-tank effluent, final sewage in treatment-plant effluent, settled sewage in Imhoff-tank effluent, and settled sludge in the bottom of the Imhoff tanks. Sept. 25, Oct. 30, 1952, Nov. 22, 1954. (SEC 1512, 1658, 2228, 2237, 2243).

Yellow Springs, Ohio. One strain was isolated from final sewage in the treatment-plant effluent. May 29, 1952. (SEC 1278).

The six cultures agree well with the standard description of this species. None fermented glucose, and all utilized ethanol as a sole carbon source. Three of these cultures formed a thin smooth pellicle on wort; the others did not, although one formed islets and one a discontinuous film on the liquid.

Trichosporon capitatum Diddens & Lodder

Dayton, Ohio. Isolated from slime scraped off surface stone on a low-rate trickling filter. April 1, 1954. (SEC 3006A).

The culture assigned to this species comes near the standard description in most cases. It did not assimilate galactose and it used ethanol well. There was good production of blastospores but arthrospores were formed sparsely.

Trichosporon cutaneum (deBeurm., Gougerot & Vaucher) Ota

Dayton, Ohio. Fourteen cultures were isolated from raw sewage in detritus-tank effluent, settled sewage from the primary settling tank, slime scraped from stone on the surface of a low-rate trickling filter, detritus, Imhoff-tank scum, medium-dry sludge, air-dry sludge, and vacuum-filter cake. July 17, 1952, Jan. 30, Feb. 12, Mar. 19, Apr. 14,

1953, April 1, 1954, Nov. 5, 1956, Mar. 28, 1955, and Feb. 11, 1958. (SEC 1286, 1287, 1803, 1805, 1884, 1885, 1886, 2041, 2097, 2108, 2117A, 2961, 3032B, 3104).

Yellow Springs, Ohio. Two strains were isolated from raw sewage in the influent, and from activated sludge in the final aerator. Oct. 9, 1952. (SEC 1557, 1564).

Ithaca, New York. Five isolates were obtained from raw sewage in the influent to the plant, and from settled sewage in the plant effluent. Sept. 2, 1952. (SEC 1377, 1386, 1389, 1394, 1410).

Lytle Creek, Ohio. One culture was isolated from water and sediment in a pool in the lower zone of recovery, Station 4.2. Mar. 27, 1952. (SEC 284).

Snow Creek, Ohio. Another single isolate was obtained from water in a stream carrying acid coal-mine wastes, Murray City, Hocking Co. April 10, 1954. (SEC 2168).

Lawrenceburg, Indiana. Isolated from grey material growing on the bank of a small tributary of Tanner's Creek carrying raw effluent from a pharmaceutical production plant. June 5, 1952. (SEC 1288).

This isolate differs from the standard description in that it assimilates galactose and maltose weakly. Its growth characteristics agree well with those described in the standard description.

The 24 isolates listed above have sufficient characteristics in common to be considered members of this species. They differ only slightly from the standard descriptions. No. 1389 assimilated galactose weakly, No. 1557 assimilated maltose weakly and No. 1288 assimilated both of these sugars weakly or latently. Most of these strains were able to utilize ethanol as a sole source of carbon, except No. 2097, and Nos. 284 and 1289 grew only weakly on it. In 30 days they usually filled the liquid wort medium with repeating pellicles. However, No. 3104 did not produce a pellicle and several produced smooth pellicles in contrast to the usually coarsely corrugated or wrinkled type of growth.

Trichosporon cutaneum var. *multisporum* (Gochet) Lodder & Kreger-van Rij

Dayton, Ohio. Isolated from raw sewage in a late stage in the pre-aeration tank, and from medium-dry sludge. Aug. 14, 1952, Nov. 5, 1956. (SEC 1329, 2932).

This variety is separated from the species by the well developed to abundant production of spherical blastospores. Two cultures which answer this description have been assigned here, but No. 2932 grows

well in the presence of nitrite as a sole nitrogen source and it is thought that it might form a transition between *T. cutaneum* and *T. pullulans*.

Trichosporon margaritiferum (Stautz) Buchwald

Dayton, Ohio. Isolated from final sewage in the secondary settling basin. Oct. 30, 1952. (SEC 1664).

SEC 1664 is assigned to this species because it answers the standard description well except that it uses ethanol only weakly as a carbon source.

Trichosporon pullulans (Lindner) Diddens & Lodder

Dayton, Ohio. Isolated from settled sewage in the effluent of the primary settling basin. Feb. 11, 1958. (SEC 3075).

Ithaca, New York. Isolated from Cayuga Lake about one mile from the sewage treatment plant. Sept. 2, 1952. (SEC 1417).

Two isolates are assigned to this species which differ nutritionally from *T. cutaneum* primarily in their ability to utilize nitrate as a sole source of nitrogen. No. 3075 is also able to utilize nitrite. As in other yeasts, pellicle formation on liquid malt extract varies with the strain. No. 2041 produced a thin film while the other developed heavy cork-like plugs of tissue which varied from smooth to coarsely corrugated.

DISCUSSION

From the above list of species which have been isolated from various polluted habitats, it has been noted that certain yeasts recur again and again in many different types of habitat regardless of the extent of pollution. TABLE II summarizes this information. The genera *Candida*, *Rhodotorula* and *Trichosporon* are best represented in the samplings made to date. The perfect yeasts were found only sporadically, with *Cryptococcus* and *Torulopsis* also poorly represented. The commonest species found were *Trichosporon cutaneum*, *Rhodotorula glutinis*, *R. mucilaginosa*, *Candida parapsilosis*, and *C. tropicalis*, in that order. The great majority of the yeasts are obligate aerobes and lack the ability to ferment one or more sugars.

None of these yeasts has been reported to have caused disease, or to have been isolated from diseased tissue in man or from diseased persons in the area covered by this study. Of 20 of the listed species, one or more strains studied by Lodder and Kreger-van Rij (1952) came from human sources, usually from diseased tissues where the yeasts may have been secondary saprobes rather than primary pathogens, or from

TABLE II

NUMBER OF HABITATS FROM WHICH YEASTS HAVE BEEN ISOLATED

Species	Habitats	Lytle Creek	Acid Mine Wastes	Lawrence-burg	Dayton	Yellow Springs	Ithaca	Pullman-Moscow	Glendale	Lawrence, Mass.
Hansenula anomala	1				1(1)					
mrakii	1	1(1)								
Saccharomyces drosophilarum	1	1(2)								
heterogenicus	1				1(1)					
Schwanniomyces sp.	2	2(2)								
Candida curvata	1				1(1)					
guilliermondii	6	3(3)			1(1)	2(2)				
humicola	5				4(4)		1(1)			
intermedia	5				2(2)	3(3)				
krusei	7				4(4)	3(3)				
parapsilosis	8		1(1)		6(8)				1(2)	
pulcherrima	1				1(1)					
rugosa	4				3(3)					1(1)
tropicalis	7				6(9)	1(1)				
utilis	1				1(1)					
Cryptococcus albidus	1								1(1)	
laurentii	3				2(2)				1(1)	
Rhodotorula glutinis	15	4(4)	1(1)		5(5)	3(3)			2(4)	
graminis	1								1(1)	
minuta	2				1(1)	1(1)				
mucilaginosa	11	3(3)	2(2)		5(5)			1(1)		
rubra	1	1(1)								
texensis	1		1(1)							
Torulopsis candida	5				4(4)	1(1)				
famata	5				4(5)	1(1)				
Trichosporon capitatum	1				1(1)					
cutaneum	15	1(1)	1(1)	1(1)	8(14)	2(2)	2(5)			
cutaneum var. *multisporum*	2				2(2)					
margaritiferum	1				1(1)					
pullulans	2				1(1)		1(1)			

Total species: 30. Total isolates: 136. Numerals in parentheses indicate numbers of isolates studied from each habitat.

sputum, faeces, or skin of diseased or healthy people. Four of the strains of *Tr. cutaneum* listed by Lodder and Kreger-van Rij had been isolated originally from the sewage works of a dairy factory in England.

The sources of the yeasts isolated during the course of this study are unknown. They may be members of a natural population of organisms living in as yet unexplored habitats. They could have been introduced to sewage, polluted water, and sewage-treatment processes through the air, runoff from soils, washings from fruit and vegetable processing, or domestic sewage.

TABLE III

YEAST POPULATIONS AT YELLOW SPRINGS SEWAGE-TREATMENT PLANT, OCTOBER 9, 1952

Area sampled	Habitat type	% of total fungus colonies	
		"White yeasts"	Rhodotorula
1. Influent	Liquid system	8.8	2.1
2. First aerator tank	Liquid system	13.0	2.0
3. Final aerator tank	Liquid system	13.6	2.0
4. Growth on end wall of final settling tank	Liquid system	13.8	1.7
5. Plant effluent	Liquid system	16.8	—
6. Digested sludge	Solids system	2.2	0.7
7. Medium-dry sludge	Solids system	1.3	0.4
8. Air-dried sludge	Solids system	1.6	17.3
9. ¼ mile down effluent creek		32.6	0.4
10. 1 mile down stream		30.4	0.3

TABLE III lists the "white yeasts" and *Rhodotorula* colonies obtained from ten sampling points in the small activated-sludge plant at Yellow Springs, Ohio, on Oct. 9, 1952. Values are given in percentages based on total number of yeast and mold colonies observed following techniques described in detail in 1954. TABLE IV gives the same type of data based on samples obtained at Dayton on Nov. 5, 1956. It should be noted that in each location all samples were obtained within a two-hour period so that, for instance, samples of liquid sewage leaving the filters were not necessarily from the same mass of sewage sampled as it entered the plant. These samplings record only the populations present at a particular point at the moment of sampling without reference to any other point.

Reference to Table 3 in Cooke and Hirsch (**1958**) indicates that, of the fungi found in continuous-sampling studies, "white yeasts" composed 17% of the total population during 57 weeks on a low-rate filter at Dayton and 27.9% on a high-rate filter, while *Rhodotorula* represented 7.9% and 9.1% respectively of the total population.

Both sets of data quoted above were obtained in a general survey of fungi during which culture techniques were no more critical for yeasts than for fungi like *Subbaromyces splendens* Hesseltine (Hesseltine, **1953**). This fact indicates that in addition to a potentially larger population of yeasts than has been developed so far, a larger number of more fastidious species may be expected to be present.

TABLE IV

YEAST POPULATIONS AT DAYTON SEWAGE-TREATMENT PLANT, NOVEMBER 5, 1956

Area sampled	Habitat type	% of total fungus colonies	
		"White yeasts"	Rhodotorula
Raw sewage	Liquid system	13.3	2.1
Detritus	Discard material	17.6	5.0
Pre-aerated sewage, late stage	Liquid system	10.0	1.6
Settled sewage	Liquid system	2.5	1.7
Filter influent	Liquid system	15.2	2.4
Filtered sewage—high rate	Liquid system	1.0	—
Filtered sewage—low rate	Liquid system	1.2	—
Final settling basin	Liquid system	11.5	5.8
Plant effluent	Liquid system	1.7	0.6
Slime, high-rate trickling filter	Permanent population	13.0	2.4
Slime, low-rate trickling filter	Permanent population	26.0	7.6
Settled sludge	Solids system	5.9	2.1
Digested sludge	Solids system	9.1	1.5
Air dry sludge	Solids system	2.5	38.5
Heat dry sludge	Solids system	—	—

The presence of a large population of organisms in such a habitat indicates that at least some of them are making use of some of the nutrients available in the polluting substances. In this way it may be assumed that some of the "wild" yeasts are aiding in sewage purification both in streams and in sewage-treatment processes. In addition, in air-drying sludge, there is an apparent increase of colonies of *Trichosporon* and *Rhodotorula* species which may be related to later stages of the dewatering process, increased aeration of the substrate, or an increasing availability of nutrient sources.

SUMMARY AND CONCLUSIONS

Thirty species of yeasts (comprising 136 isolates) have been isolated and identified from sewage, polluted water, and sewage-treatment processes.

With the techniques used, no primary pathogenic yeasts have been

found to date although 20 of the listed species have been associated with disease conditions of man, especially in the tropics.

It is suggested that yeasts find the sampled habitats adequate for carrying on growth and reproduction, and that they may contribute to sewage purification.

The isolated yeasts contained almost no perfect species. *Trichosporon cutaneum, Rhodotorula glutinis, R. mucilaginosa, Candida parapsilosis,* and *C. tropicalis* were the species isolated most frequently. Two strains of a new species of *Schwanniomyces* were isolated. The great majority of the isolates were obligate aerobes.

ACKNOWLEDGMENTS

Gratitude is expressed to Dr. Emil Mrak, Chairman, Department of Food Technology, for courtesies extended to Dr. Cooke by his laboratory and staff in the initial phases of the identification work, and for suggesting that he observe at Davis the techniques used in yeast classification. Preliminary studies on the cultures were undertaken by Dr. Knapp, whose work was supplemented by continuing studies by Drs. Shifrine and Miller. Clarification of generic and specific concepts in the more difficult mycelial and pseudomycelial genera were effected by Drs. Miller and Phaff. The overall project was supervised by Dr. Phaff.

L. T. Hagerty, Department of Water, City of Dayton, Ohio, and Drs. Graham Walton, M. J. Foter, and W. M. Ingram of the Sanitary Engineering Center have read the manuscript and contributed to the clarity of various technical statements.

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LIGHT AND THE DEVELOPMENT OF PORIA AMBIGUA

WILLIAM J. ROBBINS AND ANNETTE HERVEY

(WITH 5 FIGURES)

In the course of investigations on the nutrition of some of the Basidiomycetes, it was noted that a culture of *Poria ambigua* Bres. from Ross W. Davidson, his number 86357, formed basidiospores in the light but produced few or none in the dark. The influence of light on the development of many fungi is well known and an extensive literature on the subject exists which will not be reviewed here. Our preliminary observations indicated that *Poria ambigua* in its response to light might be an especially favorable subject for investigation.

Methods and Materials.—The basal medium [1] employed contained mineral salts, dextrose, casein hydrolysate, various purine and pyrimidine bases and the known B vitamins. The hydrion concentration was approximately pH 4.5. Cultures were grown in triplicate or quintuplicate at 25° C in 20 × 100 mm petri dishes containing 20 ml of medium, solidified with 1.5 per cent Difco agar or in 125 ml Erlenmeyer flasks which contained 25 ml of liquid medium. Our standard inoculum was obtained from colonies which had grown three or four days at 25° in the dark. Inoculum, unless otherwise stated, consisted of bits of mycelium 1 mm or 5 mm in diameter taken 3 or 4 mm from the edge of the colony by the method described by Yusef (**1953**). After inoculation, the dishes were wrapped individually in aluminum foil and incubated in a light-tight incubator at 25°. Unless otherwise indicated, the light to which cultures were exposed was a mixture of diffuse daylight and artificial light totaling from 200 to 280 foot-candles (ft-c).

Natural Materials and Growth.—*Poria ambigua* grew in our basal medium. However, growth of the fungus as measured by dry weight

[1] The basal medium contained per liter 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 20.0 g dextrose, 2 g casein hydrolysate, 809 mg adenine sulfate, 2.22 mg cytosine, 4.11 mg guanine HCl, 2.72 mg hypoxanthine, 0.25 mg thymine, 2.24 mg uracil, 0.06 mg xanthine, 5.58 mg choline Cl, 0.62 mg orotic acid, 0.337 mg thiamine HCl, 0.376 mg riboflavin, 0.205 mg pyridoxine, 0.123 mg nicotinic acid, 0.476 mg calcium pantothenate, 0.137 mg para-aminobenzoic acid, 216.19 mg *m*-inositol, 1.0 mg folic acid, 0.01 mg biotin, 0.01 mg vitamin B_{12}, 0.005 mg B, 0.02 mg Cu, 0.1 mg Fe, 0.01 mg Ga, 0.01 mg Mn, 0.01 mg Mo and 0.09 mg Zn. The casein hydrolysate was "vitamin free," from General Biochemicals, Inc. and was neutralized with $CaCO_3$.

was materially increased by the addition to this medium of natural materials such as yeast extract, tomato juice, V-8 juice, or malt extract (FIG. 1). For example, the dry weight produced in seven days in 25 ml of the basal medium containing yeast extract was as much as five times that formed in the basal medium to which no yeast extract was added.

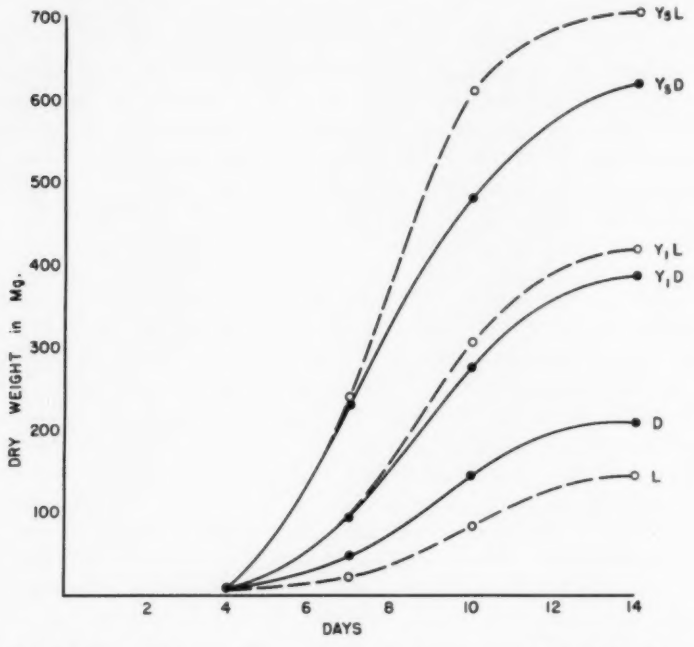

FIG. 1. *Poria ambigua*. Dry wt triplicate cultures grown in daylight (L) and in darkness (D) in basal medium and in basal medium plus 100 mg of yeast extract (Y_1) or 500 mg of yeast extract (Y_5) per culture (25 ml). In basal medium growth in dark exceeds growth in light. With sufficient yeast extract growth in light exceeds growth in dark.

Growth in colony diameter on the basal medium solidified with agar was also increased by the addition of natural materials (FIG. 2).

We believe that *P. ambigua* suffers from a partial deficiency for an unidentified growth substance (or substances) which is not included among the constituents of the basal medium but is present in the natural materials. This assumption appears justified because we have prepared a concentrate from yeast extract which had a measurable effect on growth when 10 μg were added to 25 ml of basal medium.

For convenience, we will refer to this unidentified growth substance, which may be multiple, as (y). We assume that growth in the basal medium is limited by the ability of the fungus to synthesize amounts of (y) adequate for maximum growth. The amount available to the fungus is increased by the addition of natural materials to the basal medium.

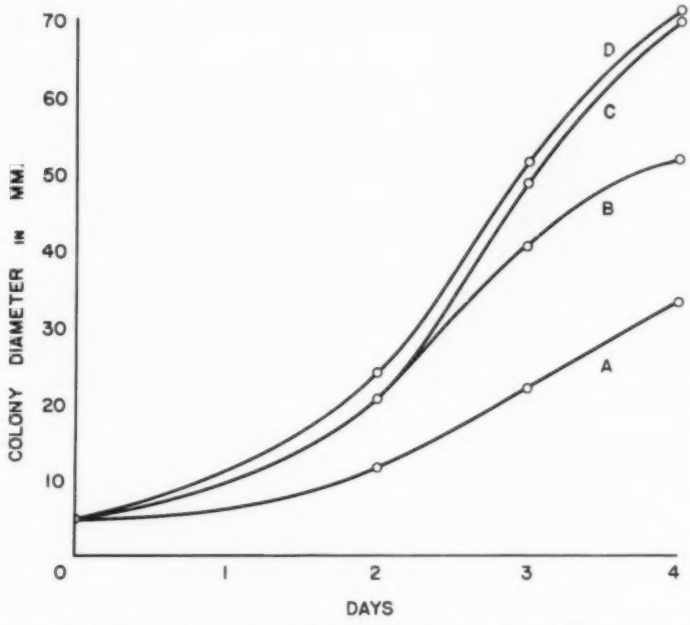

FIG. 2. *Poria ambigua.* Average diameter for 5 colonies started from 5 mm inoculum, conditioned or not conditioned and not exposed to light after inoculation. A, C—inoculum conditioned 30 min to laboratory light; B, D—inoculum not conditioned; A and B—basal medium; C and D—20 per cent V-8 agar. Note conditioning reduced growth on basal agar but had little effect on V-8 agar where growth was greater. No basidiospores were formed on A, B, C, or D. Measurements made by removing duplicate sets on the 2nd, 3rd, and 4th days.

Light and Growth.—The growth of *P. ambigua* is affected by light. The dry weight of mycelium grown two weeks in diffuse daylight in the liquid basal medium was about 75 per cent that of mycelium grown in the dark at the same temperature (TABLE I). This difference largely disappeared if yeast extract was added to the basal medium.

In the dark, growth with the larger amounts of yeast reached a maximum beyond which further additions of extract were ineffective or some-

what inhibitory. In the light, growth was further increased by the larger amounts of yeast extract (TABLE I). Tomato juice, and a highly active concentrate prepared from yeast extract, had a similar effect.

Growth in diameter on the basal agar medium was also decreased by light, and this inhibitory action was overcome by the addition of V-8 juice (FIG. 2). We did not observe, however, greater growth in diameter in the light than in the dark on the addition of the larger amounts of V-8 juice or yeast extract.

TABLE I

PORIA AMBIGUA. DRY WEIGHTS OF TRIPLICATE CULTURES GROWN TWO WEEKS IN LIGHT AND IN DARK IN 25 ML OF BASAL MEDIUM PLUS YEAST EXTRACT. GROWTH IN BASAL MEDIUM IN DARK EXCEEDS GROWTH IN LIGHT. THIS IS REVERSED WITH INCREASING AMOUNTS OF YEAST EXTRACT. SIMILAR RESULTS WERE OBTAINED WITH TOMATO JUICE AND A HIGHLY ACTIVE CONCENTRATE PREPARED FROM YEAST EXTRACT

Yeast extract mg per flask	Dry wt per 3 cultures mg				Ratio dry wt in light to dry wt in dark	
	Experiment 1		Experiment 2		Experiment 1	Experiment 2
	Light	Dark	Light	Dark		
0	37	48	41	55	75	74
5	63	73	66	88	87	75
25	88	73	94	131	120	72
100	149	139	157	166	110	94
250	244	185	226	267	132	85
500	330	176	305	212	188	144

To explain the effects of light on the growth of *P. ambigua*, we assume that the amount of (y) available in the mycelium is decreased by light. This must be effected by the action of light on the synthesis of (y) by the fungus, not by direct action of light on (y), because (y) is present in such materials as yeast extract which have been subjected to light for considerable periods. We assume, further, that growth in the presence of luxus amounts of (y) is limited by a second essential metabolite, (z), the synthesis of which is favored by light. Therefore, in the presence of the larger additions of natural materials containing (y), growth in the light exceeds growth in the dark.

Light and the Formation of Basidiospores.—Light had a profound effect on the morphology of the colony of *P. ambigua*. The nature of the response depended on the particular isolation of the fungus, treatment of inoculum, the age of the culture when exposed, wavelength of light, composition of the medium, temperature of incubation and other factors. On the basal agar medium, *P. ambigua* produced in the dark a relatively

uniform undifferentiated vegetative mycelium. The maximum effect of exposure of a colony to light was the formation of a band composed of hyphae tightly organized in a labyrinthine hymenium (FIGS. 3, 4). The band of hymenium was bordered on each side by a relatively heavy growth merging into a sparser development of vegetative mycelium. If the dishes in which the fungus was grown were inverted during growth, a spore print developed on the inner surface of the cover of the dish.

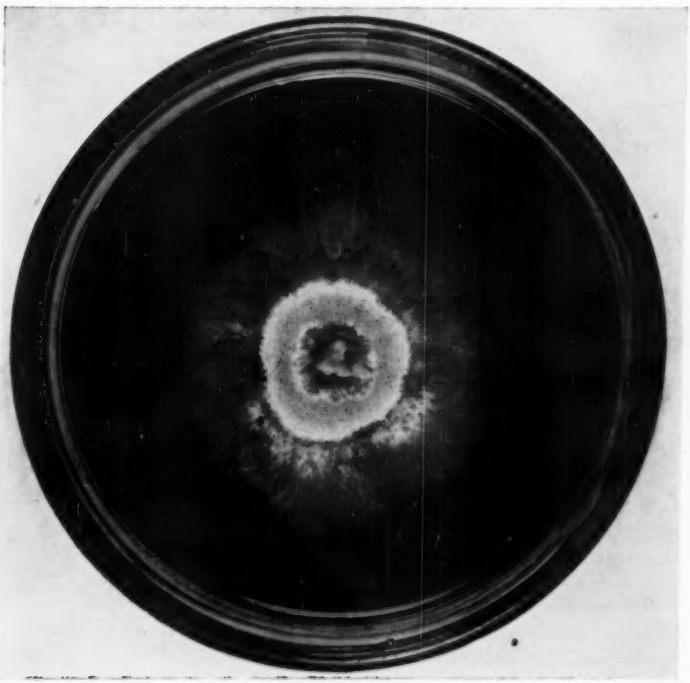

FIG. 3. *Poria ambigua*, grown on basal agar medium for 9 days. Inoculum conditioned for 30 minutes and colony exposed 30 min to laboratory light on 2nd and 3rd day after inoculation. Note ring of hymenium with vegetative mycelium outside and inside hymenium ring.

The spore print was first visible 4 or 5 days after inoculation. Less marked effects of light were evidenced by a partial ring or patches of hymenium and corresponding spore [2] deposits; sometimes by what ap-

[2] The term spores in this paper, refers to basidiospores.

peared to be an abortive hymenium which produced no spores or merely by a band of heavier vegetative mycelium.

No hymenium developed in liquid still cultures. Spores were found in only one of a dozen or so still cultures examined microscopically. These were observed in a nine-day old culture grown in the light in a 250 ml Erlenmeyer flask which contained 50 ml of 2 per cent agar covered with 50 ml of basal liquid medium.

FIG. 4. Enlargement of colony of Fig. 3 showing labyrinthine hymenium.

Application of Light.—Spores were produced in daylight and in continuous light. Short intermittent exposures were effective also if made at the proper intervals. For spore production on the basal agar medium, a single short exposure to light was inadequate. It was necessary to expose the inoculum to light and to give at least one additional exposure to the developing colony 48 hrs or 72 hrs after inoculation. An exposure made at 96 hrs after inoculation was not always effective.

The exposure of the inoculum to light we refer to as *conditioning*, thus distinguishing it from later *exposure* of the developing colony. Conditioning affected both the growth of the colony and its ability to

produce spores on the basal agar medium (TABLE II). Either the colony from which the inoculum was taken or a bit of inoculum as small as 1 mm in diameter could be satisfactorily conditioned. In normal laboratory practice, inoculum is always conditioned because inoculations are made in the light even though the cultures are later incubated in the dark. It is probable that the effect of light on the inoculum (conditioning) and its effect on the growing colony (exposure) are not qualitatively different but additive.

TABLE II

PORIA AMBIGUA. EFFECT OF CONDITIONING AND EXPOSURE TO LIGHT ON COLONY GROWTH AND PRODUCTION OF BASIDIOSPORES ON BASAL AGAR MEDIUM. INOCULUM CONDITIONED 15 MIN AND COLONIES EXPOSED ONE HR 3 DAYS, 4 DAYS, OR 3 AND 4 DAYS AFTER INOCULATION. NOTE EFFECT OF CONDITIONING ON COLONY DIAMETER AND SPORE PRODUCTION. FIGURES ARE AVERAGES OF QUINTUPLICATES

Exposure of inoculum to light (conditioning)	Diameter of colonies in mm			Production of basidiospores six days after inoculation
	3d	4d	6d	
no	29	—	57	no
yes	19	—	45	yes
no	29	39	46	no
yes	20	25	39	yes
no	—	43	63	no
yes	—	31	49	yes

While we customarily took inoculum from colonies grown for 3 or 4 days in the dark at 25°, conditioning of colonies from 3 to 17 days old was effective. The older colonies completely filled the petri dish and had largely or entirely ceased growth. As a matter of convenience, inoculum was conditioned for from 15 to 30 min, or even an hour, but 5 min was as effective as 30 min.

The effect of conditioning a fully developed colony which had largely or entirely completed its growth persisted in the dark for at least four days. A seven-day-old colony which completely filled the petri dish was conditioned for 30 min and was then held in the dark at 25°. Bits of inoculum were removed at daily intervals using care to give them no further light treatment. Each of the resulting colonies was exposed on the 2nd and 3rd day after inoculation. Colonies which grew from inoculum obtained four days after the culture was originally conditioned developed spores.

A growing colony also retained the effect of conditioning for some days when held in the dark. Three-day-old colonies 39 mm in diameter were conditioned for 30 min and placed in the dark. At daily intervals

inoculum was taken from the area of the original colony and from the new mycelium which had grown in the dark. Care was taken to give the inoculum no additional conditioning. The colonies which grew were exposed on the 2nd and 3rd day after inoculation. Colonies developing from inoculum removed from the area of the original colony four days after conditioning produced spores; colonies from inoculum secured from the new mycelium two days after conditioning produced spores, but inoculum from new mycelium removed three and four days after the original colony was conditioned were, with one exception, negative.

Exposing a colony to light during the first 24 hours after inoculation was as effective as conditioning the inoculum. If the first exposure (conditioning) was omitted (for the inoculum or the young colony), short exposures 48 hrs after inoculation or later were ineffective. At least two applications of light for colonies on our basal agar medium were required for spore production; one applied within the first 24 hrs (conditioning) and a second 48 hrs or 72 hrs after inoculation. Best spore production was obtained if conditioned inoculum was exposed 48, 72 and 96 hrs after inoculation.

An occasional colony which had been conditioned during the first 24 hrs but not exposed later, or one exposed to light for the first time 48 hrs after inoculation, produced spores. Such exceptions were infrequent and may have been caused by failures in technique.

Although a single short exposure was ineffective for colonies grown on our basal medium, one exposure 48, 72 or 96 hours after inoculation of colonies grown on 0.2 per cent malt agar from unconditioned inoculum resulted in spore production. No hymenium developed on 0.2 per cent malt agar and spore production was sparse. This difference is probably accounted for by the poorer nutritional value of 0.2 per cent malt agar as compared to our basal agar medium.

Sensitivity to Light.—P. ambigua is extremely sensitive to light. We have not determined the lower limits for either intensity or duration. However, colonies grown in diffuse daylight at 0.5 ft-c produced spores. For colonies incubated in daylight a single pinhole in the aluminum foil in which the dishes were wrapped furnished sufficient light to induce spore formation. Half or more of the colonies in dishes wrapped in a single sheet of aluminum foil and incubated in daylight developed spores because of the fortuitous leakage of light through the foil. In fact, so sensitive is *P. ambigua* to light that exposure (conditioning) of a piece of inoculum 1 mm in diameter for 30 min reduced the average diameter of the resulting colonies grown in the dark and measured 4 days later

to 70 per cent of that of colonies which had developed in the dark from similar inoculum not exposed to light (FIG. 2).

The reduction in growth of the conditioned inoculum might have resulted from a delay in initiation of growth or from a decrease in rate of growth. We believe the latter explanation is correct because we were unable to detect any difference in development between conditioned and unconditioned inoculum during the first 24 hrs after inoculation. Furthermore, during the first two or three days the slope of the curve of diameter plotted against time was less steep for the conditioned than for the unconditioned inoculum.

Excellent and uniform results were obtained when colonies were grown from inoculum, conditioned for 5 min and exposed to light for 5 min on the 2nd, 3rd, and 4th day after inoculation, a total exposure of 20 min (TABLE III). Exposure on only one of these days resulted

TABLE III

PORIA AMBIGUA. INOCULUM CONDITIONED 5 MIN. COLONIES EXPOSED 5 MIN ON 2ND, 3RD, AND 4TH DAYS AFTER INOCULATION, ONE 2ND AND 3RD DAY, OR NOT EXPOSED. NOTE SPORE PRODUCTION FROM A TOTAL OF 20 MIN EXPOSURE TO LIGHT

Light treatment	Colony diameter mm				No. colonies out of five with basidiospores after 7 days
	2d	3d	4d	7d	
Exposed	11	17	23	40	5 (4 heavy spore rings)
Exposed	11	17	—	43	5 (2 heavy spore rings)
Not exposed	—	—	—	80	0

in spore formation by some, but not all, colonies of a series; exposure on the third day was more effective than on the 2nd or 4th day.

Other fungi have been reported to be extremely sensitive to light. Medelin (**1956**) found exposure of *Coprinus lagopus* to white light for 1 sec at 250 ft-c or 5 sec at 0.1 ft-c induced fruiting. Bisby (**1925**) reported 0.25 sec of outdoor light was sufficient to induce *Fusarium discolor sulphureum* to sporulate.

Effective Wavelengths.—Light at the blue end of the visible spectrum was effective; the red end of the spectrum was not effective. Cultures illuminated continuously with a Wratten OA Safe Light produced no spores, and in growth habit resembled those grown in the dark. The Safe Light transmitted wavelengths of 540 mμ and longer with a peak at 580 mμ. The intensity of illumination was 0.4 ft-c, adequate, we believe, from other observations, to induce spore production if effective wavelengths had been used. By using the Wratten Safe Light in a

dark room, it was possible to make inoculations and handle cultures without obtaining effects due to exposure to light. Exposure of the inoculum to red light at 23 ft-c for two hrs and of the resulting colonies on the 2nd and 3rd day after inoculation was ineffective. A similar treatment with white light at 26 ft-c for 30 min periods induced the production of basidiospores. Light of short wavelength has been frequently reported to be beneficial, even essential, for the development of fruit bodies and spores by fungi (Lilly and Barnett, **1951**; Bünning, **1953**; Medelin, **1956**).

Humidity and Aeration.—We were unable to demonstrate any consistent differences in spore production between cultures grown in high humidity and those grown in low humidity, between cultures grown on freshly poured 1, 2, or 4 per cent agar, and those grown on agar which had been allowed to dry overnight before inoculation. Sealing petri dishes with Scotch tape reduced spore production, but we believe that the differences in the aeration of individual unsealed dishes in our experiments were not great enough to be significant.

TABLE IV

PORIA AMBIGUA. EFFECT OF CONDITIONING OR EXPOSURE AT 5° C ON PRODUCTION OF BASIDIOSPORES

Temperature for		No. cultures of 5 with basidiospores
Conditioning	Exposure	
25°	25°	5 (4 good spore rings)
25°	5°	5 (3 partial spore rings)
5°	25°	5 (4 good spore rings)
5°	5°	3 (3 partial spore rings)

Temperature.—The best spore production was obtained by incubating colonies at 25°. Growth was more rapid at 30° but spore formation was poor. Growth was less rapid at 20° and still further reduced at 15°. Spore production at these temperatures was less abundant and not as consistent as at 25°. Alternating temperatures during growth in the dark did not induce spores. Colonies were grown in the dark and subjected in one experiment in each 24 hr period to 8 hrs at 25° and to 16 hrs at 20° for 7 days; in another, 25° was alternated with 15°.

Although spore production was markedly affected by the temperature of incubation, a temperature of 5° during conditioning had little effect. A temperature of 5° during exposure reduced spore production to some extent, but did not prevent it (TABLE IV).

Plates with inoculum 5 mm in diameter were conditioned for 30 min at 5° or 25° and exposed for 30 min at 5° or 25° on the 2nd and 3rd day after inoculation. White light at 25 ft-c was used for conditioning and

exposure. Except for conditioning and exposure, the cultures were incubated in the dark at 25°. Before conditioning or exposure at 5° the cultures were kept at the low temperature for 1.5 hrs.

Temperatures of from 30 to 34° applied during conditioning or exposure to laboratory light had little influence on spore production by cultures incubated in the dark at 25°.

Leonian (**1924**) reported that the influence of light and temperature on pycnidium formation by some Sphaeropsidales was interchangeable. We found no evidence that temperature above or below 25° would substitute for light in the formation of basidiospores by *Poria ambigua*.

Transmission of Light Stimulus.—By comparing the diameter of a colony at the time of exposure to light with the dimensions of spore prints formed later in the dark, we concluded that only young mycelium (a zone 3 or 4 mm wide at the edge of the colony) formed hymenium and spores. We found further that new mycelium which grew in the dark during 8 to 12 hrs after exposure also produced spores, i.e., the effect of light extended into mycelium which grew later in the dark. The effects of exposure to light extended even further than the limits of the spore prints. It affected the character of the mycelium at the inner and outer edges of the fertile hymenium. The transmission of the light stimulus was noted for cultures grown on the basal agar medium and on 0.2 per cent malt agar.

A single exposure on the second or third day after inoculation of colonies grown on the basal agar medium from conditioned inoculum gave clear evidence for the transmission of the light stimulus. On 0.2 per cent malt agar, a single exposure 2, 3 or 4 days after inoculation also demonstrated transmission. When colonies were subjected to more than one exposure, the hymenium rarely extended into mycelium which developed in the dark after exposure, perhaps because the more extensive formation of hymenium and spores which resulted from multiple exposure exhausted material which might otherwise have moved into the new mycelium.

Fulkerson (**1955**) states that pycnidia were produced by *Physalospora obtusa* only in that part of the culture which had been irradiated. Medelin found that the light stimulus which induced fruiting by *Coprinus lagopus* was not transmitted to parts of the colony which were not irradiated.

Media.—Growth of *Poria* was more vigorous on media containing natural materials than on our basal medium. Associated with the more vigorous growth was a reduction in spore production. For example, on our basal agar medium supplemented with 20% V-8 (Miller, **1955**) few

or no spores were produced, even though the colonies were exposed to diffuse daylight. Although a 2 per cent malt agar extract was unfavorable for spore production, basidiospores were produced on 0.2 per cent malt agar. On the 0.2 per cent malt agar they were formed by mycelium which had not developed the labyrinthine hymenium noted on the basal agar medium and were produced in smaller quantities. Some strains of *P. ambigua* which failed to form basidiospores on the basal agar medium did so on 0.2 per cent malt agar or on a one-tenth dilution of the basal medium solidified with agar.

When spore production occurred on the dilute media, it was sometimes (depending on the fungus strain and its light treatment) limited to mycelium on the agar near the edge of the petri dish or even to mycelium which had grown up on the glass above the agar.

Partial Inhibition of Growth and Spore Production.—Although few or no spores were formed on 20 per cent V-8 agar, heavy hymenium and spores developed in some instances over or in the vicinity of a contaminant (*Penicillium* sp.) which had partially inhibited the growth of *Poria*. The contaminant was isolated and similar results obtained when the contaminant and *Poria* were grown on V-8 agar in the light in the same dish (FIG. 5). No spores were formed when the two were grown together in the dark.

The influence of one fungus on the production of asexual or sexual spores by another, when the two are grown in association, has usually been ascribed to some nutritional relation, i.e., products formed by the one organism were required by the other for spore production (see Lilly and Barnett). However, the effect described above was not caused by nutritional factors produced by *Penicillium* and required by *Poria* for spore production, but because *Penicillium* partially inhibited the growth of the *Poria*. This followed because spore production on V-8 agar was induced by partially inhibiting the growth of *Poria* by actidione.

Filter paper discs, 12.5 mm in diameter, were dipped in solutions containing amounts of actidione ranging from 1 μg per ml to 100 μg per ml. The discs were placed at the edge of the dishes of 20 per cent V-8 agar and inoculations of *Poria* made in the center of each dish. The dishes were incubated in faint diffuse daylight. Discs dipped in 1000 μg per ml of actidione caused marked inhibition and hymenium and spores were produced on each side of the disc (FIG. 5). The production of hymenium and spores under these conditions was best on the V-8 agar to which an excess of $CaCO_3$ (3 g per liter) had been added to the medium.

Inhibition by actidione in the dark did not induce spore production.

Its action did not replace conditioning or exposure to light. In other words, for good spore production on the V-8 agar, both light and partial inhibition of growth were necessary.

Strains of Poria ambigua.—In the experiments summarized above, the culture furnished by Ross W. Davidson was used. On occasion, the

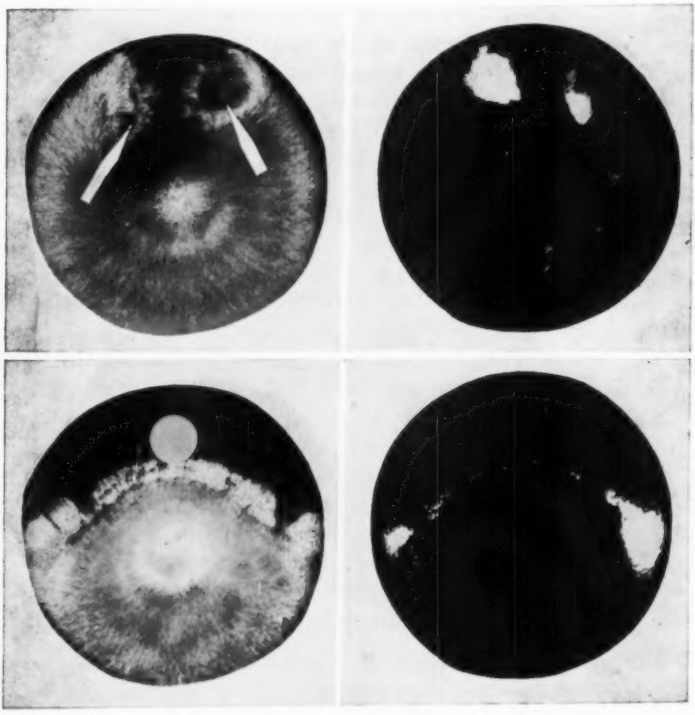

FIG. 5. Poria *ambigua*. Inhibition of growth and basidiospore production on 2 per cent V-8 agar. Upper left. Arrows point to colonies of *Penicillium* sp. which inhibited growth of *Poria*. Upper right. *Poria* spores deposited on petri dish cover. Lower left. Inhibition of *Poria* by filter paper disc containing actidione. Lower right. *Poria* spores deposited on dish cover. The spore prints are the mirror images of the colony.

results with this culture were not uniform. One or more colonies out of five treated in identical fashion failed to form spores while the others developed excellent spore prints. Various explanations for such aberrant results were considered including the possibility that we were deal-

ing with a culture which was a mixture of strains of the fungus, some fertile and some infertile, and that inoculum taken at random from a colony might consist of one or the other or both. We investigated, therefore, cultures from single basidiospores and from single hyphal tips.

With the assistance of Dr. George Bistis, 20 cultures from single spore isolates were prepared. These were tested individually and in various combinations for ability to form basidiospores. None of the single spore isolations grown on the basal medium produced spores. Some did so when grown on a nutritionally deficient medium, for example, on 0.2 per cent malt agar or on the basal medium diluted to 0.1 its normal strength and solidified with 2 per cent agar. Even on these dilute media, however, only 8 of the 20 were fertile in our hands.

Single spore isolations were grown in various combinations. Barrages developed for some combinations and it was possible to separate the cultures into two approximately equal groups according to their evidencing the barrage reaction or their failure to do so. However, no basidiospore production from the merging of two mycelia was observed.

Masses of basidiospores from the cover of a dish containing a fertile colony were used as inoculum. The colonies which developed were suitably conditioned and exposed to light, but no basidiospore production occurred. Of 25 isolates from single hyphal tips, 8 developed spores on the basal agar medium, and one equalled our stock culture in activity.

Although our experiments were not conclusive, it is probable that our stock culture was a mixture of strains varying in fertility potency. That some single spore cultures developed basidiospores on nutritionally poor media may have been because some of the spores from which the cultures were derived contained more than one nucleus. Future work on the effect of light on this organism should be based on cultures derived from single hyphal tips.

DISCUSSION

The exposure of *Poria ambigua* to light induces a complex of physiological and morphological changes. It affects the growth of the fungus as measured by colony size or by dry weight, and in our experience it is essential for the series of changes which result in the formation of hymenium and of basidiospores. It is unlikely that these effects are to be explained on any simple basis. Nevertheless, it is desirable for the direction of further investigations to make assumptions which can be tested by suitable experimentation.

In attempting to explain our observations we assume three unidentified growth substances as follows:

The first is (x), necessary for reproduction and formed on exposure to light. Evidence for the existence of (x) is based on the observation that the formation of hymenium extends into mycelium which has grown in the dark after a colony has been suitably exposed to light. This suggests that something produced by light moves into mycelium formed later in the dark. (See also effect of conditioning on inoculum taken from mycelium developed in the dark.) We assume, further, that (x) is used up in the development of reproductive structures and that it is also involved in some way in vegetative growth. The assumption that (x) is involved in vegetative growth is made because vegetatively vigorous colonies (those grown on media supplemented with tomato juice, malt extract, etc.) do not form spores except where growth is checked locally, by actidione, for example. This would permit the accumulation of (x) in that portion of the mycelium where growth is checked but not prevented. Furthermore, some isolations of the fungus which failed to form spores on our basal medium did so on a nutritionally poor medium where vegetative growth was substantially restricted.

The second is (y), necessary for vegetative growth. We assume (y) because of the growth-promoting effect of the addition of natural substances to our basal medium. On the basal medium, growth in the dark is limited by the ability of *Poria* to make this essential metabolite. On exposure to light, the fungus makes less (y) and more (x). Perhaps (x) is formed at the expense of (y). If so, a transformation of (y) into (x) is probably not involved, but rather the metabolic system responsible for (y) is diverted into that in which (x) is formed. In any event, a reduction in (y) and increase in (x) would account for the decrease in rate of growth and for the reproduction noted where the fungus is grown on our basal medium and exposed to light.

An interrelation between (x) and (y) is suggested also because the beneficial effect of light on reproduction and its detrimental effect on growth are both induced by the blue end of the spectrum and not by the red end. Furthermore, neither response is affected materially by subjecting the mycelium to low temperatures (5°) during exposure to light.

The addition of natural substances to the basal medium increased growth and interfered with reproduction. This we assume is because the natural substances supply (y) and overcome the partial deficiency for this essential metabolite from which the organism normally suffers. At the same time, since there is no direct transformation of (y) into (x), and (x) is somehow involved in vegetative growth, as well as in repro-

duction, the accumulation of (x) is inadequate for reproduction when vegetative growth is stimulated by supplying (y) in the medium.

As increasing amounts of natural material are added to the basal medium, growth in the dark increases to a maximum beyond which further additions are ineffective or become somewhat inhibitory. This, we propose, is because growth in the dark in the presence of luxus amounts of (y) is limited by (z), a growth substance the synthesis of which by the fungus is favored by light. The smaller additions of natural materials to the basal medium reduce the inhibitory action of light on growth because they supply (y). With larger additions of the natural material growth in the light exceeds that in the dark because light favors the synthesis of (z). We have not explored the possibility that (z) may be identical with (x).

Alternate hypotheses involving the formation or destruction of inhibitors might be suggested, but for the moment the assumptions presented are reasonable on the basis of our present knowledge.

Information on the cytology involved in experiments of the kind discussed in this paper would be highly desirable.

SUMMARY

Growth of *Poria ambigua* Bres. was improved by the addition of yeast extract and other natural materials to a basal medium of mineral salts, dextrose, casein hydrolysate, purine and pyrimidine bases, and B vitamins. Light reduced growth but was required for formation of basidiospores. The addition of natural materials largely eliminated the inhibitory action of light on growth, and with the addition of sufficient natural material growth in the light exceeded growth in the dark. On rich media, spore production was reduced or eliminated. Local inhibition of growth on rich media permitted spore production to occur. *P. ambigua* is very sensitive to light. Exposure of inoculum to light for a period of minutes affected growth and spore production. The light stimulus was transmitted into mycelium formed later in the dark. Some isolates prepared from single basidiospores formed spores on nutritionally poor media. Isolates grown from single hyphal tips varied in ability to form basidiospores; one equalled in potency the stock culture used in this investigation.

To explain the results, three growth substances are assumed as follows: (x) formed in the light required for reproduction but involved in vegetative growth also; (y) formation interfered with by light, required

for vegetative growth; (z) formation favored by light, limiting for growth in the dark in the presence of luxus amounts of (y).

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AN ENTEROBRYUS (ECCRINALES) IN A COMMON GREENHOUSE MILLIPED [1]

ROBERT W. LICHTWARDT

(WITH 9 FIGURES)

An undescribed species of *Enterobryus* has been found growing within the hindgut of *Oxidus gracilis* (Koch),[2] a cosmopolitan synanthropic milliped commonly inhabiting soil in greenhouse benches.

A study was made of a collection of *O. gracilis* that was supplied to me by Dr. Arthur A. Johnson who obtained them from an infested nursery in Cook County, Illinois. Previously I had attempted to find fungus infection in the same species of milliped in greenhouses on the Urbana campus of the University of Illinois, but without success.

About 80% of the Cook County population that was dissected contained at least some thalli of the *Enterobryus*. The hosts were dissected according to the method I have published (Lichtwardt, **1954**), and the fungus material was mounted on slides in lactophenol containing cotton blue.

DESCRIPTION

Enterobryus oxidi sp. nov.[3]

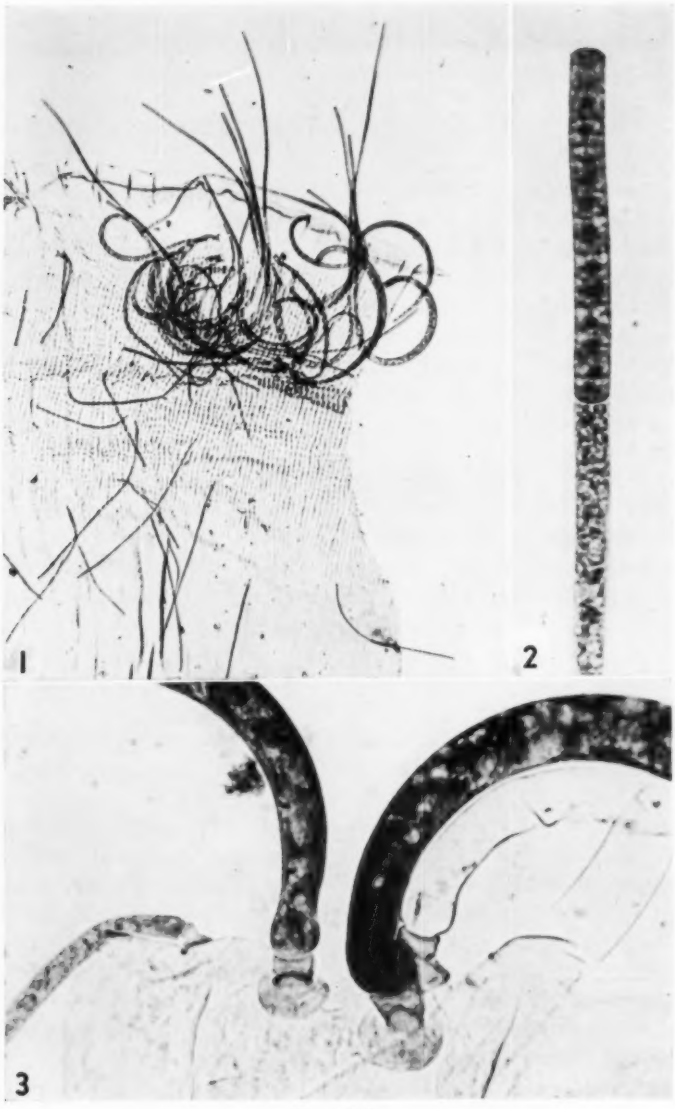
Maioribus thallis cum flexu prominenti propinqua basi. Latissimis in flexu 15–21 μ lat. cum angusto immuenti usque ad 6–9 μ lat. termino distanti; hyalinis; producentibus uninucleatas sporangiosporas circa 6–13 $\times$ 6–9 μ lat. Minoribus hyphis rectis aut propinquis basi flexis circa 5–7 μ lat. cum parvis retinaculis inaequalibus; hyalinis; producentibus uninucleatas sporangiosporas aut longas sporangiosporas multinucleatas usque ad 170 μ long. $\times$ 6 μ lat. Binucleatis sporis raris circa 60 $\times$ 26 μ. Ellipsoideis sporis praesentibus circa 18–20 $\times$ 4–5 μ.

Larger thalli with a prominent curve near the base, widest in the curved portion that measures 15–21 μ in width, tapering to about 6–9 μ distally; hyaline; producing uninucleate sporangiospores measuring about

[1] This paper is based upon a portion of a Ph.D. thesis submitted to the University of Illinois in 1954. I am indebted to Dr. Leland Shanor who supervised this research.

[2] The identification of the milliped was kindly made by Dr. Richard L. Hoffman.

[3] I am indebted to Professor Austin Lashbrook, Department of Latin and Greek, for his assistance in preparing the Latin diagnosis.



FIGS. 1–3.

FIGS. 1–3. *Enterobryus oxidi.* FIG. 1. Anterior portion of milliped hindgut lining, with wide and narrow thalli, × 68. FIG. 2. Uninucleate sporangiospores, × 600. FIG. 3. Holdfasts attached to gut lining, × 600.

6–13 × 6–9 μ wide. Smaller hyphae essentially straight, or curved near the base, about 5–7 μ wide, with small, irregular holdfasts; hyaline; producing uninucleate sporangiospores, or long multinucleate sporangiospores up to 170 μ long by about 6 μ wide. Binucleate spores rare, measuring about 60 × 26 μ. Ellipsoid spores present, measuring about 18–20 × 4–5 μ.

Attached to lining of hindgut of the milliped *Oxidus gracilis* (Koch). HOLOTYPE: slide no. *A-1-2a, R. W. Lichtwardt.*

This species of *Enterobryus* is quite distinct from any other that has been described. The most noticeable feature of the species is the presence of the curved and tapering thalli concentrated along the anterior margin of the lining of the hindgut (FIG. 1). These larger hyphae bear series of terminal uninucleate aplanospores (FIG. 2) of the kind I have previously designated as type F (Lichtwardt, **1954**). Their holdfasts are usually well-formed, with a cylindrical stalk and a wide basal disk (FIG. 3).

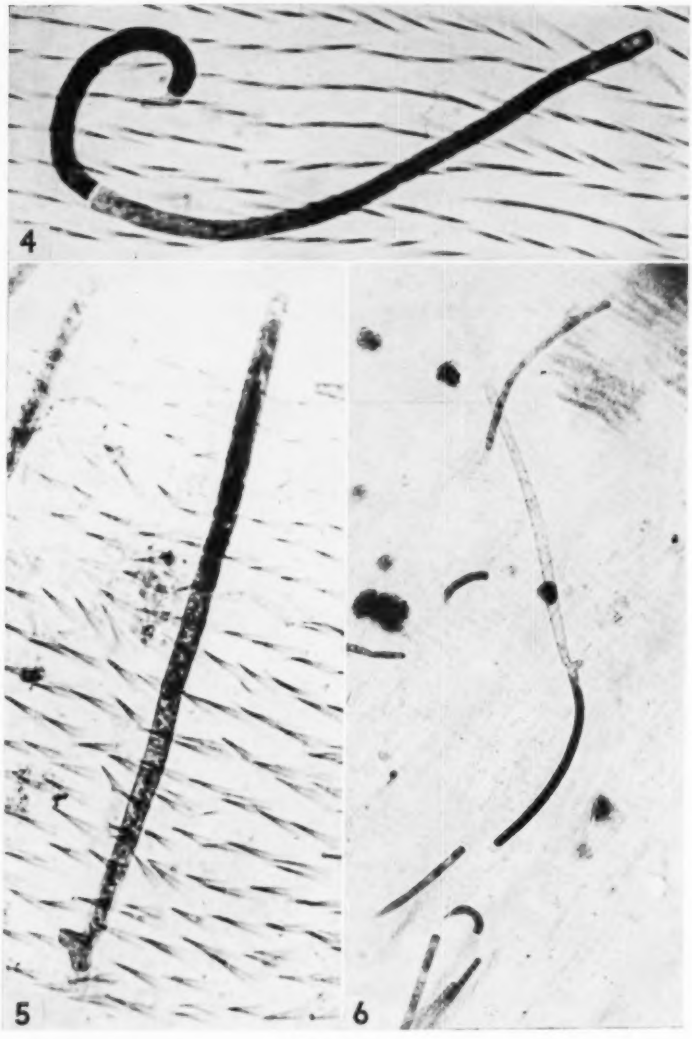
Interspersed among these larger thalli are smaller ones (FIGS. 1, 3) that measure about 6–7 μ in width, which may also produce uninucleate sporangiospores similar to those produced by the wider hyphae. More commonly, however, one finds the narrow hyphae in a position posteriad to the larger filaments (FIG. 1), and producing either uninucleate spores or long, multinucleate sporangiospores of type A (FIG. 4). The holdfasts of the narrower hyphae occasionally have a basal disk similar to, but smaller than, those found on the wider hyphae (FIG. 3), though the holdfasts frequently are quite irregular and varied in shape.

A few of the dissected millipeds were infected only with the narrower hyphae, lacking the wider, curved thalli along the anterior margin of the hindgut.

An interesting feature can be observed in the germination of some of the spores of type A. Instead of producing a holdfast at the very tip of the spore, as happens in most species of *Enterobryus,* the tip of the spore may become reflexed, and the holdfast and new hyphae then form at the hooked portion just lateral to the tip, with the spore tip protruding from the axis of the hypha in a manner that somewhat resembles a young branch (FIGS. 5, 6).

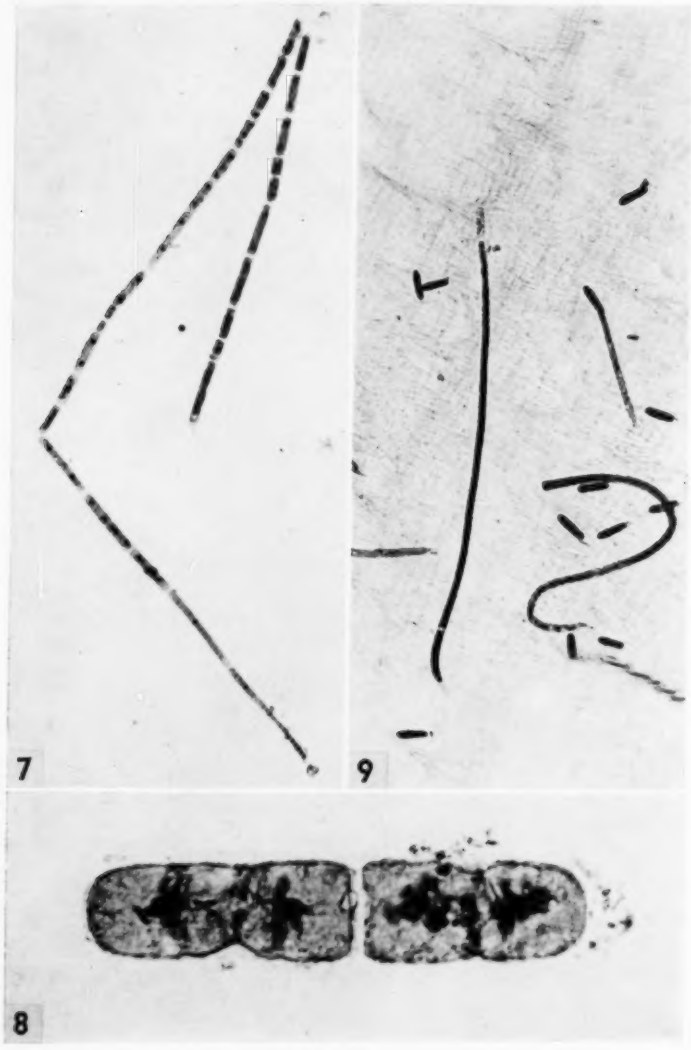
Two specimens of *Oxidus* had within their guts a few hyphae bearing binucleate spores of type D (FIGS. 7, 8), basically very similar to those I have described in *Enterobryus apheloriae* from the milliped *Apheloria iowa* Chamberlin (Lichtwardt, **1954**).

In the guts of some individuals were large numbers of ellipsoid spores of type I in various stages of germination (FIG. 9). I could find



FIGS. 4–6.

FIGS. 4–6. *Enterobryus oxidi*. FIG. 4. A short thallus with one multinucleate sporangiospore, × 600. FIG. 5. Multinucleate spore beginning to germinate, producing a slightly lateral holdfast and hypha at the hooked end of the spore, × 600. FIG. 6. Further stage in the germination, showing the characteristic small hook remaining at the base of the spore, × 260.



FIGS. 7–9.

FIGS. 7–9. *Enterobryus oxidi.* FIG. 7. A filament with binucleate spores in various stages of development, × 160. FIG. 8. Two connected binucleate spores, × 600. FIG. 9. Ellipsoid spores attached to and germinating on the gut lining, × 260.

no hyphae that were producing this spore type in the millipeds I dissected. It seems evident that the animals containing these spores had ingested them, and that the spores therefore represent an inoculum from another milliped. It is possible that these spores are formed only at one particular time in the host, such as just prior to the molting process, as is reported to happen in some other species of Eccrinales, for example, in *Eccrina flexilis* Léger and Duboscq, or *Enterobryus duboscqui* Tuzet and Manier. It is also possible that these spores are at first essentially the same as the uninucleate sporangiospores already described, but that they change to a more oval form after emergence from the sporangium, as I have observed to happen (Lichtwardt, **1957**) in *Enterobryus moniliformis* (Leidy) Lichtwardt.

DISCUSSION

An eccrinid already has been reported by Käthe Maessen (**1955**) in what is apparently the same species of milliped. (She calls the host *Orthomorpha gracilis* C. L. Koch; but see Chamberlin & Hoffman, **1958**, p. 84). The following is Maessen's Latin diagnosis of *Enterobryus flavus* (p. 119): "Vivens in proctodaeo Orthomorphae gracilis C. L. KOCH; fila dura, in inferiore parte crassiora et forma guttae, habentia pedes primo angustos forma infundibuli; fila et pedes brunneo fere colore; etiam fila pedes non habentia; multiplicatio fit ex sporis 1 nuclei aut 4 aut 8 nucleorum aut capsulis fertilibus; conjunctio seminum."

On pages 33–36 she gives a fuller description and illustrates the fungus. It is evident from both the descriptions and her drawings that her species of *Enterobryus* and the one I have treated in this paper are quite distinct. On this basis I have not hesitated to name mine as a new species, in spite of the identical host.

SUMMARY

A new fungus, *Enterobryus oxidi,* in the hindgut of the milliped *Oxidus gracilis* (Koch), is described and illustrated. Distinctly wider and narrower hyphae are formed, and four types of spores were observed. This species appears to be quite different from *Enterobryus flavus* Maessen, described from the same host in Europe.

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ANGULAR LEAF SPOT OF MAGNOLIA

A. G. PLAKIDAS

(WITH 1 FIGURE)

In October, 1958, the State Entomologist's office sent to us two leaves of *Magnolia grandiflora* L. bearing very numerous angular necrotic lesions (FIG. 1, A) for diagnosis. On the underside of the lesions there were numerous erect, compactly fasciculate, fungus fruiting structures bearing fusiform septate conidia at the apex, obviously those of *Isariopsis*. As no disease of magnolia caused by *Isariopsis* was found listed in the Index of Plant Diseases in the United States, it was thought that the spotting might have been caused by some other factor and the fungus might be a secondary invader of the injured leaf tissues.

About one week later, the nursery in which the original specimen was collected (Folsom, St. Tammany Parish, Louisiana) was visited. There were about 1,000 young magnolia plants growing in containers (quart or gallon cans) ranging from about 6 to 18 inches in height, and all were diseased in varying degrees. Some, especially among the older, larger plants, were very severely affected (FIG. 1, A), while others had relatively few spots. According to the owner, the disease was first noticed early in June, and it became progressively worse as the summer advanced. The plants were seedlings, and there was considerable variability in the size, shape, thickness, and pubescence of their foliage. It was noted that infection was much heavier and the disease much more severe on plants with relatively thin, sparsely pubescent, leaves than on those with thick, stiff, densely pubescent leaves, and this fact was later confirmed by more detailed observations and inoculation experiments. Environmental factors undoubtedly favored infection and disease development. The plants were crowded, in partial shade (under pine trees) and watered by overhead sprinklers.

DESCRIPTION

Typically, the disease is characterized by very numerous angular necrotic spots, brown, and 1–3 mm. The necrotic areas are noticeably depressed on both surfaces of the leaf. The spots may be discrete, but, more often, they coalesce to form large necrotic areas, and the leaf assumes a dry, scorched appearance. On the under surface, the color

of the necrotic spots is dark brown; on the upper surface, the younger spots are dark brown, but the older spots have a silvery sheen due to the drying and bleaching of the epidermis. On some plants the spots

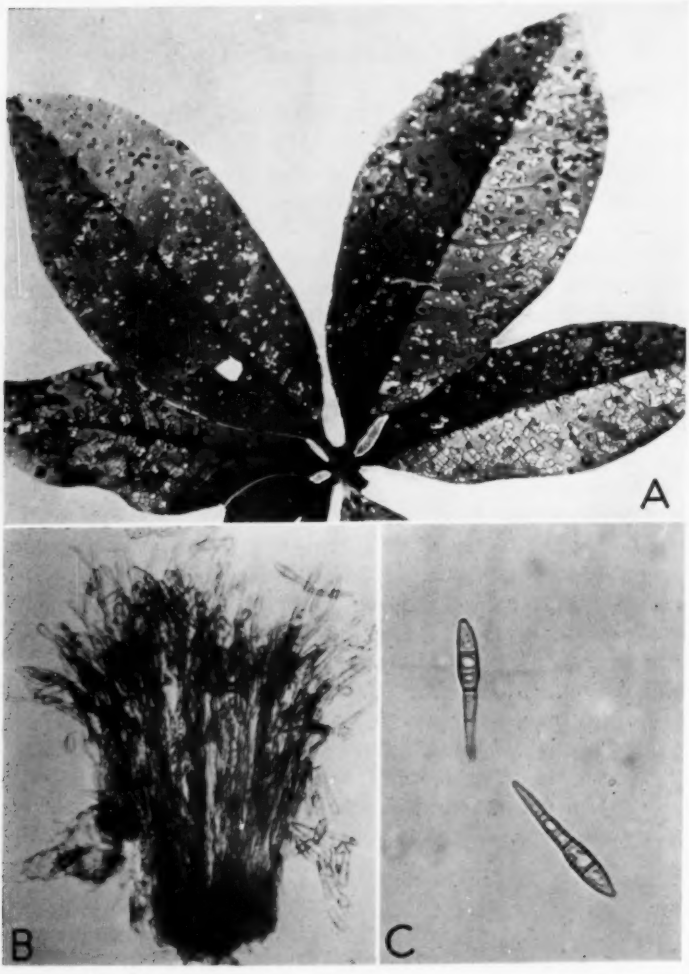

FIG. 1. A. Young *Magnolia grandiflora* plant severely affected with angular leaf spot. Approx. ½ nat. size. B. Synnema and conidia 145×. C. Conidia 700×. (By coincidence, both these conidia are 3-septate, but the conidia of this fungus are predominantly 2-septate.)

are more circular than angular, somewhat larger than the typical angular spots, and frequently surrounded by a yellowish halo. The under surface of the necrotic lesions bears very numerous sporophores (synnemata) and conidia typical of *Isariopsis* (FIG. 1, B, C).

ORIGIN OF THE DISEASE

This disease appears to be new and undescribed. No reference to a disease of this type on magnolia has been found in pathological or mycological literature. The disease was found in several nurseries in Southeast Louisiana (Tangipahoa and St. Tammany parishes). It was particularly severe on young plants in lath houses and in the field. On older plants (4 to 10 years old and 5–8 feet tall) infection was generally light, although an occasional heavily spotted and partially defoliated plant was encountered. A survey in the woods in the vicinity of these nurseries failed to find the disease on magnolias (*M. grandiflora* L. and *M. virginiana* L.) growing in the wild, so it does not appear likely that the infection is indigenous. One of the nurserymen stated that he had observed the disease for the first time 10 years earlier on small magnolia plants he bought from another nursery in Forest Hill (Rapides Parish, in central Louisiana). However, a careful inspection of the Forest Hill nurseries in 1959, first by the writer and later by the district nursery inspector failed to reveal the disease in this area. The origin of the disease, therefore, remains unknown.

IDENTITY OF THE FUNGUS

The causal fungus fits well within the genus *Isariopsis* Fries. Its fruiting structures are typical synnemata (FIG. 1, B). No reference to *Isariopsis* on magnolias or related hosts has been found in the literature. The fungus is therefore described as a new species. Specimens of two other species of *Isariopsis, I. griseola* Sacc. on bean and *I. clavispora* (Berk. & Curt.) Sacc. on grape, were available for comparison with the magnolia fungus. The bean specimen consisted of fresh material (bean leaves) kindly supplied by Dr. D. C. Arny, University of Wisconsin, and this made it possible to obtain the fungus in culture and test its pathogenicity on magnolias. Repeated parallel inoculations were made with the bean and magnolia isolates on magnolia (*M. grandiflora* L.) and on bean (*Phaseolus vulgaris* L. 'Black Valentine'). Clear-cut results were obtained from these cross inoculations. The magnolia isolates infected the magnolias but not beans, and, conversely, the bean isolates infected beans but not magnolias. There were also cultural and

morphological differences between the bean and the magnolia fungi. The former sporulated abundantly on autoclaved beanpods; the latter failed to sporulate on this medium. The conidia of the bean fungus were fusiform, curved, predominantly 3-septate, and averaged 6.6 × 57.7 μ in size. The conidia of the magnolia fungus were obclavate, straight or curved, predominantly (over 90%) 2-septate, and averaged 6.6 × 37.1 μ in size. These morphological, cultural, and host differences make it clear that the magnolia *Isariopsis* is different from *I. griseola*.

The *I. clavispora* specimen was dry herbarium material (grape leaves) collected at Lake Charles, Louisiana in 1923, and was not suitable for isolation or inoculation. The conidia on this specimen were fusiform, curved, predominantly (over 80%) 3-septate, and averaged 5.9 × 55.3 μ in size, thus differing from those of the magnolia fungus in size, shape, and septation.

The magnolia *Isariopsis* was isolated, both from tissue plating and from single conidia, and grown on 3 different media, potato dextrose agar, autoclaved beanpods, and autoclaved magnolia leaves plus 1 per cent sucrose solution. On potato dextrose agar the colonies were raised, greenish gray on top, black on reverse, small (average diameter of 12 two-week-old colonies 11 mm). Sporulation on this medium was sparse and the conidia were produced not on synnemata but on a felty stroma of long, loose, brown conidiophores. On autoclaved beanpods the growth was more luxuriant than on potato dextrose agar, but no sporulation occurred. On autoclaved magnolia leaves the colonies developed slowly at first and were greenish gray and compact, but, in about a month, the medium became covered with light gray aerial mycelium. Sporulation was very abundant on this medium, and the conidia were produced on well-formed but somewhat loose synnemata.

Isariopsis magnoliae sp. nov.

Maculae angulares, necroticae, subtus atrobrunneae, supra brunneae ad colorem argenteum vergentes, 1–3 mm lat. Fructificatio hypophylla; synnemata stipitata, brunnea, 42–70–104 μ crass., 168–302–490 μ long.; conidia obclavata vel fusoidea, dimidii basalis diam. dupla apicalis, extremis ambobus rotundatis, recta vel arcuata, subhyalina, 1–3- (plerumque 2-) septata, 6.2–7.0 × 28.0–56.0 (medio 37.1 × 6.6) μ.

Spots angular, necrotic, dark brown on the under surface, brown to silvery on the upper surface, 1–3 mm in size. Fruiting hypophyllous; synnemata stipate, brown, 42–104 (ave. 70) μ in thickness and 168–490 (ave. 302) μ long; conidia obclavate to fusoid, the basal half approximately twice the diameter of the apical half, base and apex rounded, straight or curved, subhyaline, 1–3-septate, predominantly 2-septate, 6.2–7.0 × 28.0–56.0 (ave. 37.1 × 6.6) μ.

HOST: *Magnolia grandiflora* L.

TYPE LOCALITY: Folsom, Louisiana, November 4, 1958, *A. G. Plakidas,* No. 8241.

DISTRIBUTION: In several nurseries in Folsom and Loranger, Louisiana.

TYPE specimens, No. 8241, deposited in the mycological herbarium of the Department of Botany, Bacteriology, and Plant Pathology, Louisiana State University, Baton Rouge, Louisiana, and also in the National Fungus Collections, Bureau of Plant Industry, Washington, D. C. and the herbarium of Cornell University, Ithaca, New York.

PATHOGENICITY

The disease was reproduced in its typical form in 6 separate experiments in the greenhouse by inoculating potted magnolia plants with conidial suspensions of pure cultures of the fungus. Prolonged exposures to a highly humid atmosphere were necessary for heavy infections. When the plants were kept in the moist chamber for 5 days following inoculation, very heavy infection (thousands of angular spots) resulted; a shorter exposure (2 to 3 days) resulted in comparatively light infection. The young, fully expanded but still tender leaves were markedly more susceptible than the older, fully hardened leaves.

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THE FUNGUS CAUSING PECKY CYPRESS

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(with 7 figures)

In a preliminary report on the cause of pecky decay of bald cypress, *Taxodium distichum* (L.) Rich. (**5**), it was stated that basidiocarps of a *Stereum* had been collected from this host and that cultures derived from spores of this *Stereum* were similar to cultures isolated from typical pecky decay. In the present paper this *Stereum* is described as a new species and compared with the better known *S. sulcatum* Burt, which it closely resembles.

Several specimens of the cypress *Stereum* have been collected in the past by pathologists interested in cypress decay. Von Schrenk obtained a specimen, apparently from a cypress log, in Louisiana in March 1898. This is the specimen reported from Louisiana by Burt (**2**) as *S. sulcatum*. Long collected specimens of the same species on a cypress log in Texas in May 1918. Neither Long (**16**) nor von Schrenk (**29**) indicated that this *Stereum* might be connected with pecky cypress. One other specimen was collected by A. S. Rhoads near Gainesville, Florida, in December 1943. These three basidiocarp specimens and the one recently collected in Mississippi (**5**) suggest that the fungus is widespread on cypress in the South. Collections made since this study was started indicate that the species is common in mature cypress stands.

The *Stereum* on cypress is especially interesting because its basidiocarps resemble those of *S. sulcatum;* however, on more careful examination, differences are apparent. Cultures of the two are quite distinct; those of *S. sulcatum* obtained from hosts such as Engelmann spruce, *Picea engelmannii* Parry, and hemlock, *Tsuga canadensis* (L.) Carr., show no resemblance to cultures of the species on cypress. These differences are detailed in the following descriptions.

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[2] Mycologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

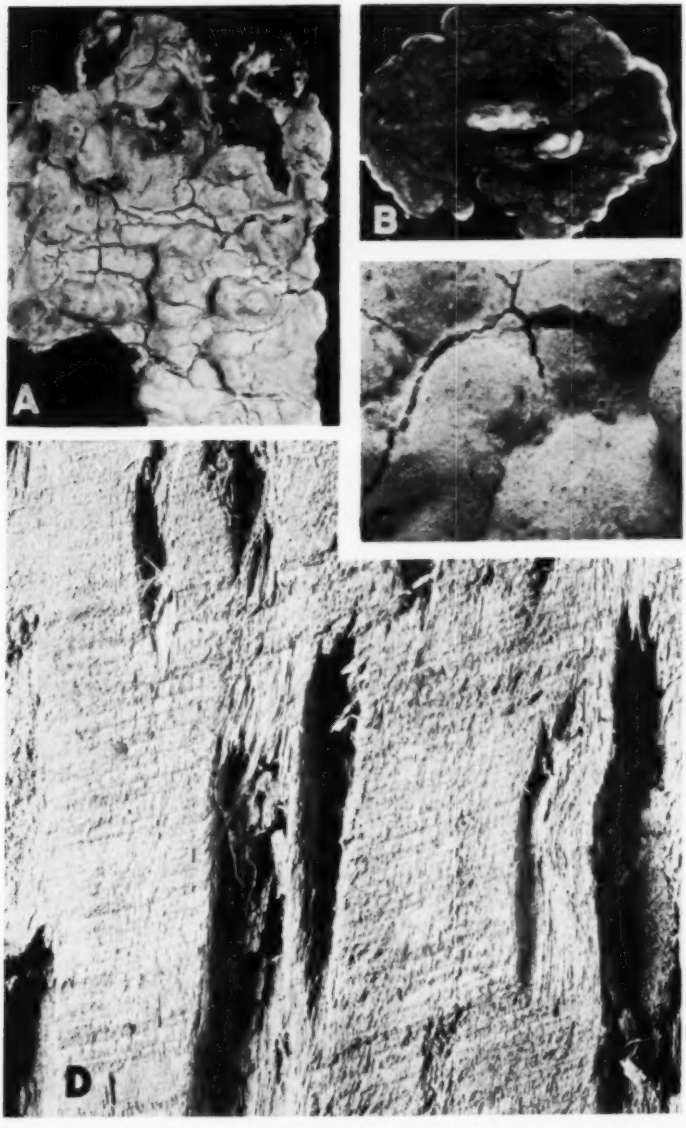

FIG. 1. *Stereum taxodii.* A, Hymenial surface of basidiocarp, × 2; B, Upper surface of basidiocarp, × 2; C, Portion of hymenial surface, × 10; D, Typical pecky cypress decay.

Stereum taxodii Lentz & McKay, sp. nov.[4] [FIGS. 1; 2, B–D; 3; 4, B, D, F; 5, B; 6, B]

Basidiocarpus lignicola, superficie superiori umbonibus minutis affixa, pileo plerumque reflexo, 0.5–1 mm crassa vel in partibus nonulis multo crassioris; superficies superioris velutina, brunnea, deinde glabra, distincte sulcata et fusca; superficies hymenialis griseoris quam pallide ochraceo-salmonea vel obscurioris quam pallide lutea; tomentum usque ad 250 μ crassum, supra compactum, rufo-brunneum; contextus subhyalinus, ex hyphis semi-parallelibus, crasse tunicatis, 4–7 (−8.5) μ in diametro, et hyphis inconspicuis, ramosis, septatis, fibuligeris, 2.5–5.5 μ in diametro compositis; cystidia apicibus incrustatis, 65–80 × 7–14 μ; basidia circa 50–75 × 8 μ; sporae glabrae vel verrucoso-tuberculatae, tunica amyloidea, tuberculis valde amyloideis, 6–8.5 × 5.5–7 μ.

Macroscopic Characteristics: Basidiocarps lignicolous, pliant when thin, but thickened areas hard and rigid, the margin free from the substratum or more often distinctly and broadly reflexed, with various areas of the superior surface attached to the substratum by umbos or by narrow attachment ridges (FIG. 1, B); the superior surface at first velutinous, or appearing to be almost tomentose under magnification, rather indistinctly sulcate, "Argus brown"[5] to "Brussels brown" or intermediate between "Verona brown" and "warm sepia" (approximating *Montella*), later thickening and becoming hard and glabrous, distinctly sulcate, "fuscous," with the margin sometimes becoming much thickened and very deeply sulcate; the hymenial surface dull and dry, smooth or slightly uneven, sometimes with delicate cracks in dried specimens (FIG. 1, A, C), the color always characterized by a perceptible grayish cast, slightly grayer than "pale ochraceous-salmon" (resembling *sombrero*), or somewhat darker than "light buff" (near *maple* or closely resembling *putty* and *ecru-beige*).

Microscopic Characteristics: In section (FIG. 2, B) the unmodified portion of the pileus approximately 0.5–1 mm thick, becoming as much as 4 mm thick or probably thicker in modified areas through the umbos or through stratified pilei; the tomentum approximately 50–250 μ thick in the specimens examined, reddish brown or brownish, originating from the superior surface of the pileus; the superior surface consisting of a dense hyphal layer well characterized by a deep reddish brown color approximating "Hay's russet" or "vinaceous-russet"; the context subhyaline or pale yellowish, under low or medium magnification appearing to consist of thick-walled hyphae rather liberally sprinkled with particles of incrusting matter, with the hyphae sinuously semiparallel, more or less horizontal in the upper central region of the context (FIG. 2, C), deviating toward the superior surface from the upper side of this region

[4] Latin description prepared by Edith K. Cash.

[5] Colors given in quotation marks are from Ridgway (**27**), and those in italics from Maerz and Paul (**17**).

and toward the hymenial surface from the lower side; the hymenial region usually characterized by crystalline cystidial apices quite distinct from the less prominent hyphal apices.

Hyphal system dimitic; the skeletal hyphae (FIG. 3, A) very conspicuous in the context, non-septate or with only very thin secondary

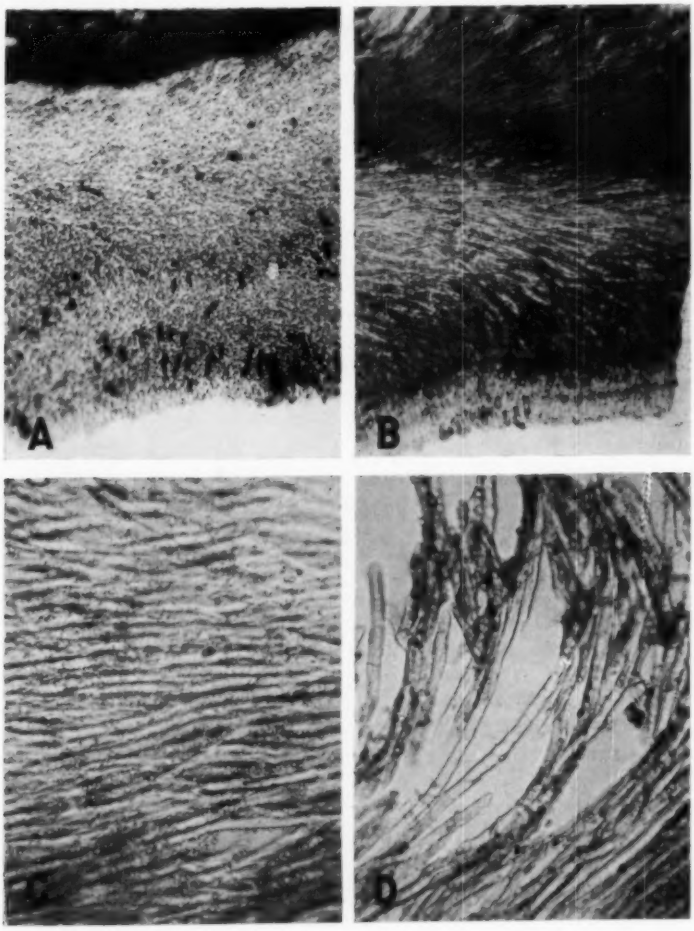

FIG. 2. Photomicrographic section of basidiocarps. A, *Stereum sulcatum*, × 70; B, *S. taxodii*, × 70; C, Portion of section of basidiocarp of *S. taxodii* showing upper central context composed of heavy skeletal hyphae, × 310; D, Tomental hyphae of *S. taxodii*, × 310.

septa, lacking clamp connections, unbranched, 4–7(–8.5) μ in diameter, with walls usually at least 1 μ thick and often with some portions of the hyphae becoming completely solid, the skeletal hyphae also appearing as colored, thick-walled tomental hyphae (FIG. 2, D); the generative hyphae (FIG. 3, B) hyaline, branching, with clamp connections, 2.5–5.5 μ in diameter, with walls as much as 0.5 μ thick, the generative hyphae appearing in all parts of the basidiocarp but obscured by the much more conspicuous skeletal hyphae.

Cystidia (FIG. 3, C) usually abundant or occasionally somewhat infrequent in the simple hymenium of thin basidiocarps, appearing at various levels in the modified subhymenial regions of thickened basidiocarps, some protruding approximately 10 μ beyond the hymenial surface, others immersed as much as 225 μ or perhaps deeper beneath the hymenial surface, each cystidial apex consisting of a dense crystalline mass covering the terminal portion of a hypha, with both the hyphal tip and the crystalline sheath rounded at the apex, the cystidial pedicels breaking off in slide mounts of crushed material and the cystidia then approximately 65–80 $\times$ 7–14 μ, the hyphal tip under the incrustation approximately 5–7 μ in diameter, the apical incrustation approximately 16–32 μ long, with the incrusted apices of submerged cystidia appearing to be generally longer than those of superficial cystidia; basidia (FIG. 3, D) each characteristically subtended by a clamp connection, 4-sterigmate, approximately 50–75 $\times$ 8 μ; spores (FIG. 3, E) appearing at the hymenial surface and also submerged throughout the thickened subhymenial regions of modified pilei, non-septate, subglobose, smooth-walled or minutely echinulate, hyaline, amyloid, 6–8.5 $\times$ 5.5–7 μ, the smooth spores having a tendency to be somewhat smaller than the verrucose spores.

SPECIMENS EXAMINED: Florida-Gainesville (coll. *A. S. Rhoads*, det. H. S. Jackson as *S. sulcatum*); Louisiana-Lutcher (coll. *H. von Schrenk*, det. E. A. Burt as *S. sulcatum*); Mississippi-Stoneville (coll. *E. R. Toole, F. P.* 105284); Texas-Sabinal (coll. *W. H. Long*, F.P. 30571, TYPE). All specimens of *S. taxodii* have been collected from *Taxodium distichum* and are filed in the National Fungus Collections.

Diagnostic Features: Although the basidiocarp of *Stereum taxodii* seems remarkably similar to that of *S. sulcatum*, critical comparison of the two species reveals dissimilarities in a number of details. As noted by Lentz (**13**) the hymenial surface of *S. sulcatum* produces an illusion of having been fashioned from delicately salmon-tinted chalk. This bright aspect of the hymenium is so unusual among *Stereum*-like fungi that it may be looked upon as a principal identifying characteristic of this species. In contrast, the hymenial surface of *S. taxodii* has a grayish shade that is noteworthy when compared with the brighter hy-

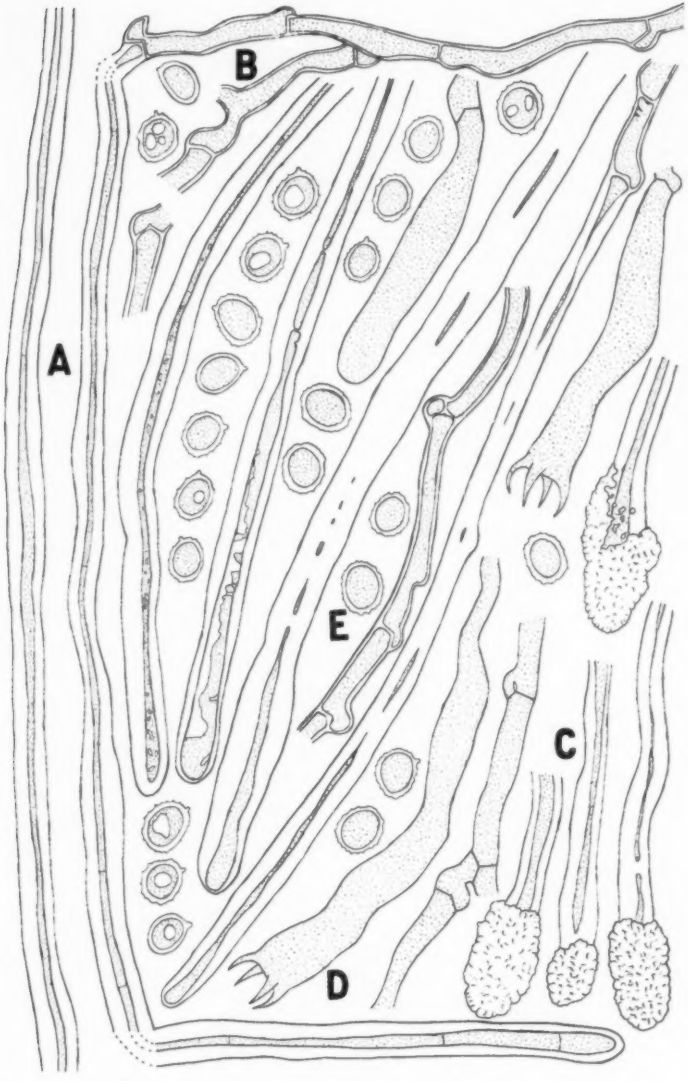

FIG. 3. Camera lucida drawings of basidiocarp elements of *Stereum taxodii*. A, Thick-walled skeletal hypha; B, Generative hypha; C, Cystidia; D, Basidia; E, Basidiospores, × 1000.

menium of *S. sulcatum*. This color difference is associated with differences in the morphology of the two species. In *S. taxodii* the generative hyphae have little significance as structural units of the pileus. Instead, the predominating elements in the context are the abundant skeletal hyphae. These are easily recognizable as they form a pattern of semiparallel, thick-walled elements when seen in a vertical section of the pileus. In *S. sulcatum* no such pattern is evident, for the hyphae seem to be closely entangled and without especial orientation. In this species, skeletal hyphae are relatively infrequent in proportion to the abundance of generative hyphae. Many of the generative hyphae are thin-walled and have evident clamp connections. But much more important in determining the configuration of the context are the innumerable, relatively slender, thick-walled hyphae (FIG. 7, B) that branch profusely but with the branches usually remaining relatively short. These hyphae seem to have rare clamp connections and thus are thought to be generative hyphae that remain slender but have greatly thickened walls. These determine the orientation of all the hyphae of the context, for they tend to grasp and bind all the other hyphae so that even the skeletal hyphae are interlaced in the hyphal aggregation without definite orientation. The context elements of *S. taxodii* may be separated to a considerable degree by pulling the skeletal hyphae apart and thus forming cleavage lines, but the context of *S. sulcatum* does not cleave and may only be torn apart. In the tomentum of the two species this same pattern of hyphal characteristics appears, that of *S. taxodii* being formed primarily by skeletal hyphae whereas that of *S. sulcatum* consists principally of generative hyphae. The hyphae in the tomentum of *S. sulcatum* may be broad and thick-walled, but they have clamp connections.

The spores of *S. taxodii* (FIG. 3, E) and *S. sulcatum* (FIG. 7, E) are alike in general aspect, but those of *S. taxodii* are slightly larger, on the average, than those of *S. sulcatum*. This is also true of basidial and hyphal dimensions, although the difference is not enough to be of considerable help in characterizing the two species. For the latter purpose, the dissimilarities in hyphal relationships and cultural characteristics exemplify the fundamental differences between *S. taxodii* and *S. sulcatum*.

DESCRIPTION OF CULTURES

Key Pattern: B-O-S-1-2-11-16 and A-O-S-1-2-10-14.[6]

[6] The methods used in studying the cultures and the arrangement of the descriptions and explanation of the key pattern are those used in previous studies in this laboratory (**4**). Petri dish cultures for mat descriptions and growth rates were incubated at 25° C on Fleischmann's diamalt agar. Test-tube cultures were grown at room temperature (about 25° C) in diffuse light. The test for oxidase reaction follows the methods described by Davidson et al. (**3**).

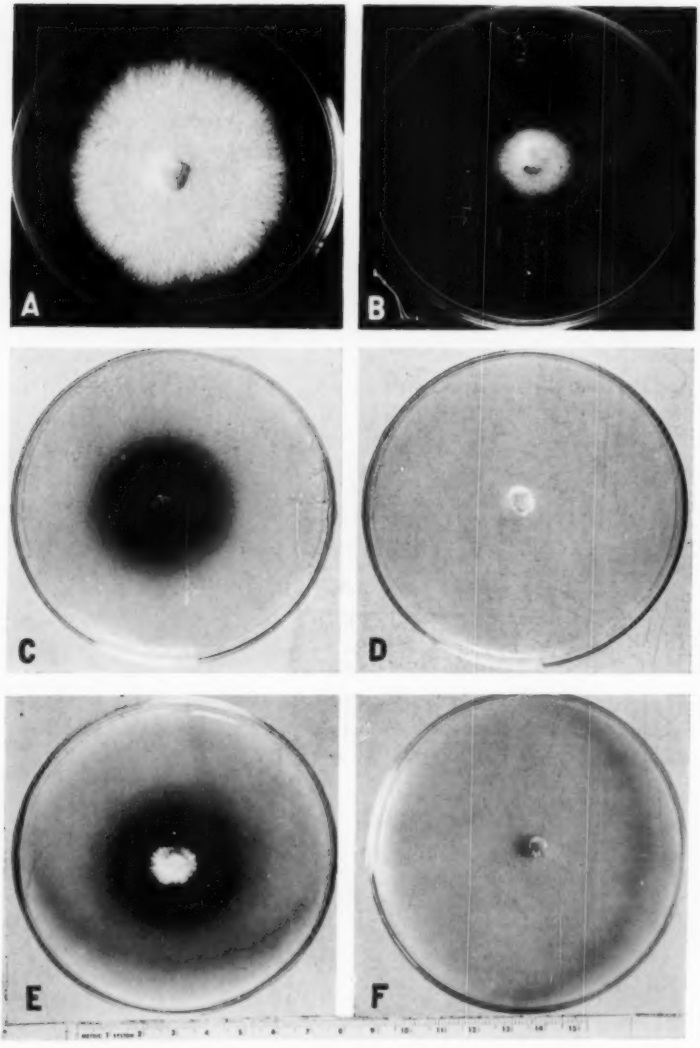

FIG. 4. A, C, and E, *Stereum sulcatum* cultures, A, growth on malt agar at 14 days, C, gallic acid agar at 7 days showing strong oxidase reaction, E, tannic acid agar at 7 days showing slight growth and strong oxidase reaction; B, D, and F, *S. taxodii* growth on B, malt agar at 14 days, D, gallic acid agar at 7 days, F, tannic acid agar at 7 days, the latter two showing negative oxidase reaction.

Growth Characteristics: Growth on 2.5 per cent diamalt agar slow, varying from a trace to 24 mm in diameter in 7 days and 22–48 mm in 14 days (FIG. 4, B); the mat fragile at first, then friable in central region, raised-woolly over the inoculum block, with the central area around the inoculum block compact-downy, floccose to pulverulent, or loose-woolly,

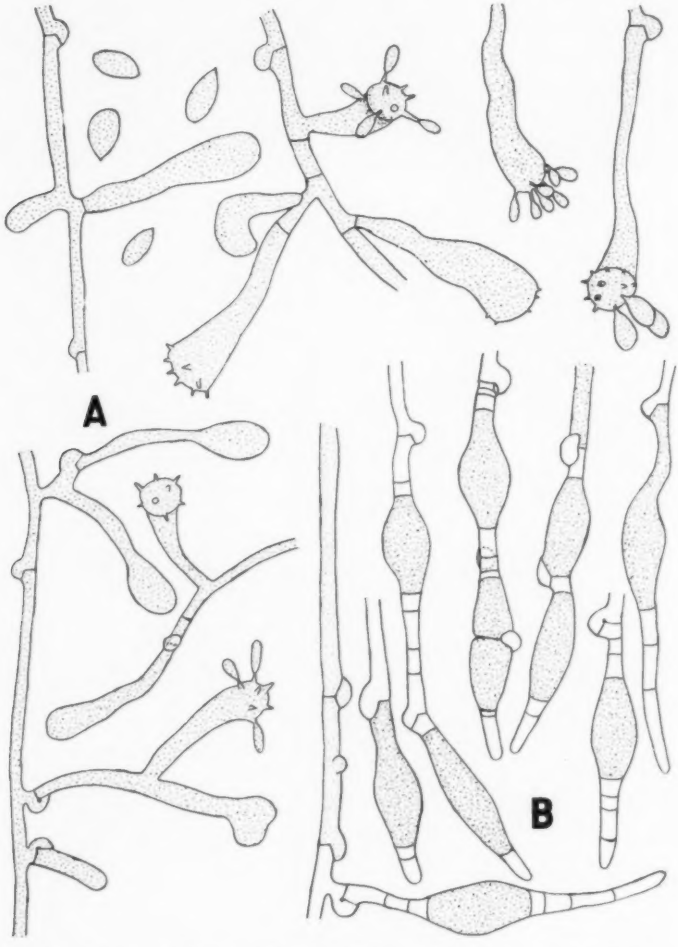

FIG. 5. Camera lucida drawings of diagnostic elements from cultures. A, *Stereum sulcatum* showing conidiophores and conidia; B, *S. taxodii* showing hyphae segments with chlamydospores attached, × 1000.

gradually diminishing in thickness from the center to the thin, translucent marginal region, with the margin appressed and delicately fimbriate; the color of the mat at first white, then "light buff" with a "light drab" tinge in the central region, the color increasing in intensity as the mat ages, becoming "cinnamon-drab" or "drab" within 2–4 weeks, often with the dark stain of the inoculum block visible through mat; the underside of mat "light drab" to "hair brown" in the center at 14 days, becoming "benzo brown" to "dusky brown" within 3–4 weeks;

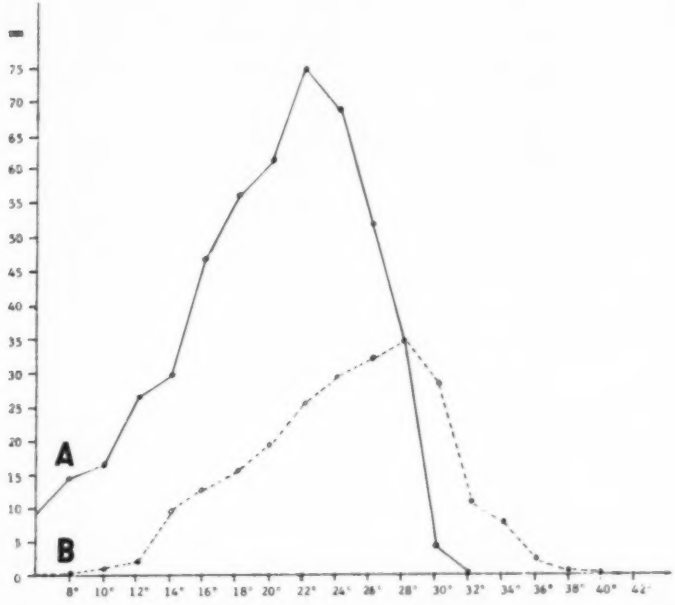

FIG. 6. Average diameters of mats in millimeters against constant temperatures in centrigrade used over a 14-day growth period for A, *S. sulcatum* and B, *S. taxodii*.

medium around the mat frequently stained "olive-yellow"; culture odorless; the oxidase reaction usually negative on both gallic acid and tannic acid media (FIG. 4, D, F), or occasionally with a stain sufficiently dark to be called a positive reaction, the mycelium exhibiting a trace of growth on gallic acid medium, no growth on tannic acid medium.

In test-tube cultures at 28 days the mat covering slant and upper portion of diamalt agar cylinder with compact-downy to felty growth, gradually diminishing in thickness to thin translucent margin; the mat "pale

olive-buff" or "tilleul buff"; agar under inoculum region "benzo brown," with surrounding area light "pecan brown."

Microscopic Characteristics: Staining hyphae (1.5–)2–3(–6) μ in diameter, with many large, simple clamp connections, and with numerous short hyphal branches originating at right angles; fiber hyphae hyaline, thick-walled, 2.5–6 μ in diameter, common in older mats (14–21 days), sometimes lacking at 14 days, frequently terminating in enlarged pseudocystidial tips 3–7 μ in diameter, with lumina expanded at the hyphal apices; chlamydospores numerous (FIG. 5, B), terminal or intercalary, lemon-shaped or oblong-ellipsoidal, 12–25 × 4–10 μ, frequently in pairs or chains when formed from adjacent hyphal cells, the walls slightly thickened, hyaline; incrusted hyphae rare or absent, occasionally present in center of mat at 14 days.

Temperature Relations: Seventeen isolates of *S. taxodii* were plated in triplicate on diamalt agar in Petri dishes and grown in the dark at constant temperatures ranging, at two degree intervals, from 8° to 46° C. The average diameters of the mats in millimeters at 14 days are shown in FIG. 6, B. The minimum temperature, at which only a trace of growth occurred, was 10°, the optimum 28°, and the maximum for growth 38°. At 40° all cultures failed to show even a trace of growth, but after transfer to diamalt agar tubes and incubation at 25° for 14 days all cultures grew. After incubation at a temperature of 42° for two weeks 41 per cent grew upon return to favorable conditions and 10 per cent were able to survive at 44°. But after two weeks at 46° none grew when transferred to fresh tubes.

Cultures Studied: The cultures of *S. taxodii* used in preparing the description of cultural characteristics are those from *Taxodium distichum* listed in the paper by Davidson et al. (**5**) and several from more recent collections. The following were selected for study: Florida-Lake City (F.P. 48278-R [7]); Georgia-Jesup (F.P. 105215-R, F.P. 105316-R), Valdosta (F.P. 105210-R, F.P. 105211-R); Mississippi-Benoit (F.P. 106256-Sp), Scott (F.P. 105464-Sp), Stoneville (F.P. 105284-Sp, F.P. 105284-R, F.P. 105318-R); North Carolina-Elizabethtown (F.P. 105317-R).

Remarks: In culture this fungus is easily recognized by the slow-growing, compact, downy mat with "light drab" tinge, by the negative or very weak oxidase reaction with no growth on tannic acid but a trace of growth on gallic acid medium, by the numerous chlamydospores, and by the large fiber hyphae terminating in expanded pseudocystidial tips.

[7] R = culture obtained from wood decay; Sp = culture obtained from germinated spores.

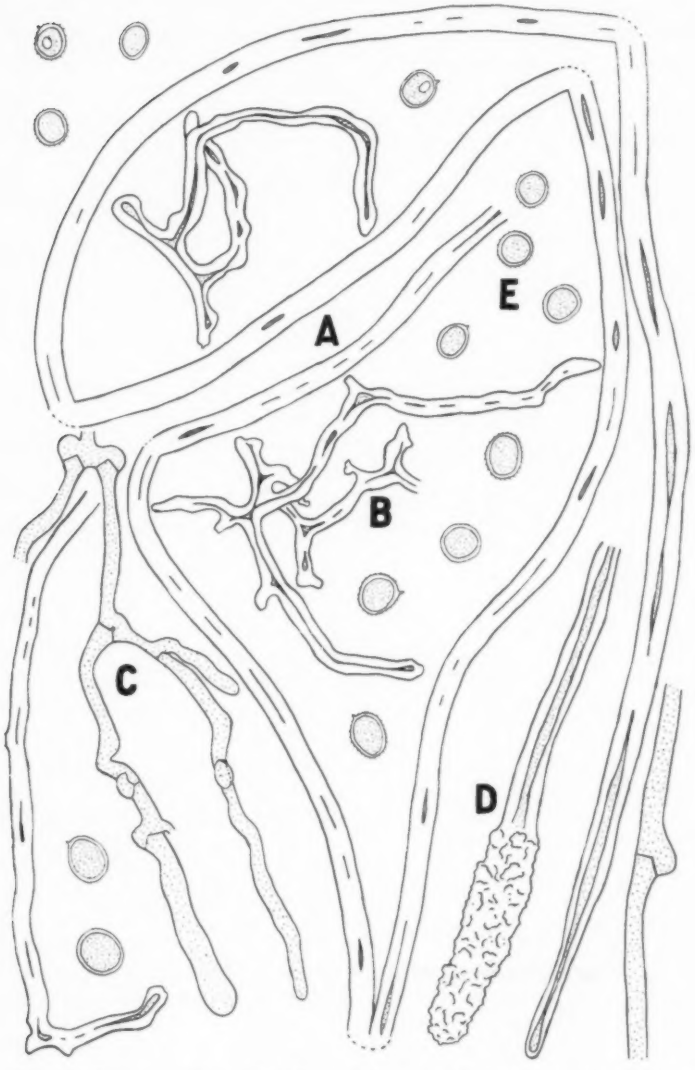

FIG. 7. *Stereum sulcatum* basidiocarp elements drawn by camera lucida. A, Thick-walled skeletal hypha; B, Branched thick-walled generative hyphae; C, Branched thin-walled generative hyphae; D, Cystidium; E, Basidiospores, × 1000.

Type of Decay: Causing a white stringy to brown powdery rot in the form of elongate pockets (FIG. 1, D) scattered through the heartwood, as described by Davidson et al. (**5**).

STEREUM SULCATUM Burt in Pk., N.Y. State Mus. Ann. Rpt. 54: 154. 1901. [FIGS. 2, A; 4, A, C, E; 5, A; 6, A; 7]

S. sulcatum is well characterized by descriptions given by Burt (**2**), Eriksson (**7**), Lentz (**13**), Litschauer (**14**), Overholts (**24**), Pouzar (**26**), and several other authors. Critical features of the species include the minutely echinulate spores, relatively slender thick-walled hyphae, nodose-septate tomental hyphae, and brightly colored hymenium.

DESCRIPTION OF CULTURES

Key Pattern: B-P-M-1-3-11-16 and A-P-M-1-3-11-16.

Growth Characteristics: Growth moderately vigorous on 2.5 per cent diamalt agar, forming a mat (20–)30–40(–47) mm in diameter at 7 days and (42–)55–75(–88) mm at 14 days (FIG. 4, A); the mat adherent, firm to tough in the center, fragile in the marginal area, growth at first loosely floccose-woolly, becoming compacted in the center by 14 days, forming a mound over the inoculum block and spreading outward in cottony strands, often faintly zonate and occasionally marked with indistinct radiations, raised in the marginal region, with margin fimbriate; the color of the mat at first white, then with only a trace of color or becoming "light ochraceous-buff" or "pinkish cinnamon" in the central zone and with a more or less extensive white marginal zone, later becoming "cinnamon-buff" in the center, with the margin remaining white; the underside showing no color change, or more frequently with a "pinkish cinnamon" tinge; culture having a faint fruity odor; the oxidase reaction strongly positive on both tannic and gallic acid media (FIG. 4, C, E), with no growth on gallic acid medium, moderate growth on tannic acid medium with the mat similar to that on malt but smaller and frequently with spreading silky fans of growth in marginal zone.

In test-tube cultures at 28 days the mat covering the slant and extending to base of cylinder in some tubes or about half the length of diamalt agar plug in others, ending in a straight, raised, even margin; the mat thickly floccose-woolly to downy or farinaceous, "light buff," with some areas "vinaceous-cinnamon," "tawny," or "light pinkish cinnamon"; agar under the inoculum region "tawny" to "Mikado brown," with surrounding area faint "pinkish cinnamon."

Microscopic Characteristics: Staining hyphae 1–4 μ in diameter, with few branches and abundant simple clamp connections; fiber hyphae (1.5–)2(–3) μ in diameter, with thick, refractive, hyaline walls, aseptate, rarely branched, usually present at 14 days and abundant within 21 days; pseudocystidial tips of fiber hyphae long, slender, 2–3 μ in diameter, with

lumina expanded at apices but without increase in hyphal diameters; enlarged hyphal tips scattered in center of mat at 14 days, conspicuous within 21–28 days, deep-staining, with few to many knob-like or verrucose protuberances, the walls at first hyaline, becoming thickened and brown, with contents including many oil globules; oedocephaloid conidiophores abundant (FIG. 5, A), formed as enlarged clavate hyphal tips with subtending clamp connections, 16–25 × 6–10 μ, narrowing to 2–3 μ in diameter at the bases, with numerous tapering sterigmata at the apex of each conidiophore; conidia hyaline, smooth, fusiform-ellipsoidal or obpyriform, apiculate, 7–10 × 2.5–4 μ.

Temperature Relations: Following the method described for *S. taxodii* 18 isolates of *S. sulcatum* were used in making a temperature study (FIG. 6, A). The minimum temperature for growth of *S. sulcatum* is somewhat below 8° C, the optimum 22° and the maximum 30°. Cultures of *S. sulcatum* held at 32° and 34° for two weeks were transferred to fresh tubes of diamalt agar and incubated at 25° for 21 days. Since none of these transfers showed growth, a killing temperature near 32° is indicated.

Cultures Studied: Colorado (F.P. 94423-R, F.P. 100268-Sp, F.P. 100281-Sp, F.P. 100296-Sp, F.P. 104502-Sp, F.P. 104517-Sp, F.P. 105104-Sp, F.P. 105166-Sp, 51-35-R, all on *Picea engelmannii*, F.P. 94417-R on *Pinus contorta* Dougl., F.P. 104454-Sp on *Abies lasiocarpa* (Hook.) Nutt.); Pennsylvania (F.P. 71329-Sp, F.P. 71688-R, both on *Tsuga canadensis*); Tennessee (F.P. 86355-R on *T. canadensis*).

Remarks: Although basidiocarps of *S. sulcatum* are superficially similar to those of *S. taxodii* in appearance, the two species are very dissimilar in cultural characteristics. Cultures of *S. sulcatum* develop warm shades of cinnamon or buff color, have a strong positive oxidase reaction with good growth on tannic acid medium and no growth on gallic acid medium, produce an abundance of fusiform-ellipsoidal conidia on oedocephaloid conidiophores, fail to form chlamydospores, and have a temperature range of 8° to 30°, with an optimum of 22°. Our culture studies of *S. sulcatum* confirm those made by Maxwell (**18**), except that growth is more rapid under the conditions used in our studies. Cultures of *S. taxodii* grow more slowly than those of *S. sulcatum*, have a dull drab tinge of color, are negative in oxidase reaction or only occasionally produce a stain on gallic acid and tannic acid media, develop only a trace of growth on gallic acid medium and fail to grow on tannic acid medium, produce numerous chlamydospores but no conidia, have large fiber hyphae expanding to form pseudocystidial hyphal tips, and have a temperature range of 10° to 36° with an optimum temperature of 28°.

Type of Decay: Causing a white pocket rot to white stringy butt rot in heartwood of conifers, sometimes extending to considerable height in old infected trees.

INTERFERTILITY STUDIES

Since the study of sexuality in the Hymenomycetes by Vandendries (**28**), pairing of monosporous cultures has been found useful in the separation of confused species as shown by Mounce and Macrae (**21, 22**), McKeen (**20**), Nobles (**23**), Harmsen, Bakshi, and Choudhury (**9**), McKay (**19**), and several others. As pointed out in these studies, two basidiocarps in question are considered to belong to the same species if monosporous mycelia from the two are interfertile when grown together.

TABLE 1

RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS 13 MONOSPOROUS CULTURES OF S. SULCATUM AND 2 MONOSPOROUS CULTURES OF S. TAXODII. THE SYMBOL + INDICATES THE PRESENCE OF CLAMP CONNECTIONS AND 0 THEIR ABSENCE

		51-35-R	71329-Sp	71688-R	94417-R	94423-R	100281-Sp	100296-Sp	104502-Sp	104517-Sp	105166-Sp	104454-Sp	105104-Sp	86355-R	106256-Sp	105464-Sp
Stereum sulcatum	51-35-R		+	+	+	+	+	+	+	+	+	+	+	+	0	0
	71329-Sp	+		+	+	+	+	+	+	+	+	+	+	+	0	0
	71688-R	+	+		+	+	+	+	+	+	+	+	+	+	0	0
	94417-R	+	+	+		+	+	+	+	+	+	+	+	+	0	0
	94423-R	+	+	+	+		+	+	+	+	+	+	+	+	0	0
	100281-Sp	+	+	+	+	+		+	+	+	+	+	+	+	0	0
	100296-Sp	+	+	+	+	+	+		+	+	+	+	+	+	0	0
	104502-Sp	+	+	+	+	+	+	+		+	+	+	+	+	0	0
	104517-Sp	+	+	+	+	+	+	+	+		+	+	+	+	0	0
	105166-Sp	+	+	+	+	+	+	+	+	+		+	+	+	0	0
	104454-Sp	+	+	+	+	+	+	+	+	+	+		+	+	0	0
	105104-Sp	+	+	+	+	+	+	+	+	+	+	+		+	0	0
	86355-R	+	+	+	+	+	+	+	+	+	+	+	+		0	0
Stereum taxodii	106256-Sp	0	0	0	0	0	0	0	0	0	0	0	0	0		+
	105464-Sp	0	0	0	0	0	0	0	0	0	0	0	0	0	+	

Conversely, the failure of monosporous mycelia from two basidiocarps to pair is interpreted as indicating that they belong to two different species. In many heterothallic species, interfertility or lack of interfertility may be readily demonstrated by the presence or absence of clamp connections.

To apply this criterion, monosporous cultures of *S. taxodii* and *S. sulcatum* were grown together in all possible combinations and examined for clamp connections after 10–14 days (TABLE 1). Complete interfertility with production of clamp connections resulted when monosporous mycelia of *S. sulcatum* from thirteen basidiocarps were paired,

and the same occurred when single spore cultures of *S. taxodii* from two basidiocarps were grown together. Clamp connections were not formed in any monosporous pairings of *S. sulcatum* with *S. taxodii*. Thus interfertility pairings emphasize the lack of specific relationship revealed by morphological and cultural differences.

DISCUSSION

The cause of pecky cypress has been a subject for investigation and controversy for many years. The early study by von Schrenk (**29**) provided considerable information on host relationships, distribution, and importance of the disease, but did not identify the causal organism. A few years after these early investigations, Long (**16**) attempted, by circumstantial evidence, to determine what fungus was causing the decay. The species he collected most frequently on living cypress trees was *Fomes ulmarius* (Fr.) Gill. (as *F. geotropus* Cke.). Long concluded that this species of *Fomes* was most logically the cause of the pecky disease, despite variation in the type of decay associated with the basidiocarps and notwithstanding the fact that these were usually formed on or near the bases of trees rather than in the top or upper trunk areas where von Schrenk found most of the pecky rot. *F. geotropus* has been considered as the cause of hollow butts, especially on hardwood hosts (**12**), but Long collected it several times on cypress associated with limited decay of the so-called pecky type. Boyce (**1**) and Overholts (**25**) are among several more recent investigators who have collected fungi in the South or who have examined the evidence presented by Long. They expressed doubt that *F. geotropus* causes pecky cypress.

In Japan, Hemmi et al. (**10**) described a decay of *Cryptomeria japonica* (L.f.) D. Don as resembling the pecky decay of cypress. Basidiocarps of *F. ulmarius* were reported as associated with this decay. Studies in the Forest Disease Laboratory (**15**) reveal a distinct difference in growth temperature relationships between cultures of the Hemmi fungus and those of *F. ulmarius*. In any event, cultures of *F. ulmarius* from the United States and Great Britain are not similar to cultures obtained from pecky cypress decay.

As mentioned earlier in this paper, von Schrenk and Long collected from cypress two specimens identified, until the present investigation, as *S. sulcatum*. There is very little published information on decay of living trees by *S. sulcatum*, but it was one of the species included by Hubert (**11**) in his paper on the diagnosis of decay in wood. He lists it as occurring on several conifers, especially *Larix occidentalis* Nutt., where it causes a white pocket rot. More recently, it was reported from

spruce in Canada by Denyer and Riley (**6**) and by Etheridge (**8**). Each of these authors reported only one infected tree, and in both instances the disease was reported as a butt rot. The original description of *S. sulcatum* by Burt (**2**) was based on basidiocarps from coniferous hosts other than *Taxodium* and characterizes a fungus relatively well-known in North America. The species also occurs in Europe, and Eriksson (**7**) recently stated that it is an important element of the fungus flora in virgin forests of Muddus National Park in Northern Sweden, where it occurs on fallen trunks of *Picea*.

S. sulcatum is a common species on conifers, especially logs of *Abies lasiocarpa* and *Picea engelmannii*, in the Rocky Mountains, and has been collected on injured or dead areas at the ground line of large, overmature, living trees in that area. Although it has been reported as causing a white pocket rot, in more typical or more advanced stages it causes a white stringy rot. It has not been observed to cause distinct white pockets as do *Fomes pini* (Fr.) Overh. or *F. nigrolimitatus* (Rom.) Egeland, two associated species in the Rocky Mountain area. The decay of these various coniferous hosts by *S. sulcatum* does not resemble the pecky decay in cypress.

Although the pecky decay is not considered to be a white rot, several who have studied it carefully have remarked that it does not seem to be a typical brown rot. This agrees with our examination of specimens with which we have worked. In the earlier stages of development the pockets of decay have a white stringy to laminated appearance. In this respect it differs from the pocket rot of incense cedar (**29**), with which it has sometimes been compared. In the more advanced stages, the pockets become filled with a powdery mass of soft decay, and there are some indications of cross checking as in a carbonizing type of decay; but this feature is seldom conspicuous. This was also brought out in von Schrenk's study by his illustration of the early stage of decay in Plate 1, figure 1, and his description of the early stages of pocket formation (**29**).

The specimens collected by von Schrenk and Long and filed in the National Fungus Collections as *S. sulcatum* assumed considerably more significance after it was determined that they were misidentified and that they are identical with a basidiocarp specimen found on branch stubs of a living cypress. This specimen, F.P. 105284, collected by E. R. Toole near Stoneville, Mississippi, was growing on the trunk of a cypress tree approximately 30 feet above the ground. A culture was obtained from germinated spores of this basidiocarp, and the culture was found to be identical with cultures obtained from pecky decay. Thus both

von Schrenk and Long many years ago had collected from cypress the species we believe to be the cause of pecky decay. This species is *S. taxodii*. When the recent culture studies were started, it was felt that there might be several rather similar decays in cypress. Information up to the present disproves this. To date, five basidiocarp specimens in addition to F.P. 105284 have been collected from the tops of cypress trees by E. R. Toole. Cultures from these are also identical with those from pecky cypress decay and thus confirm our belief that this decay is caused by *S. taxodii*.

SUMMARY

The well known decay of bald cypress is now known to be caused by the newly described *Stereum taxodii* Lentz & McKay. It is similar to *S. sulcatum* Burt, common on spruce and fir in the Rocky Mountain forests and on eastern hemlock in Pennsylvania, New York, and other areas.

There are distinct differences between these two *Stereum* species in host relationships, in structure of basidiocarp, in type of decay, and in cultural characteristics. *S. taxodii* is a top rot fungus, apparently gaining entrance through branch stubs or broken tops, whereas *S. sulcatum* is a butt rot organism. The former causes a distinctive type of rot, which in typical stages consists of rather large pockets of advanced decay scattered through the heartwood in a uniform and characteristic fashion. The latter typically causes a white stringy rot, which in some stages resembles a rather irregular white pocket rot.

Growth and temperature relationships are quite different in the two species; and *S. sulcatum* has a conidial stage and no chlamydospores, whereas *S. taxodii* has no conidial stage but numerous chlamydospores. *S. sulcatum* gives a strong oxidase reaction, whereas *S. taxodii* has a very weak oxidase reaction. Detailed descriptions are given for both the species.

PLANT INDUSTRY STATION
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CULTURAL CHARACTERISTICS OF FOMES ULMARIUS AND PORIA AMBIGUA

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(WITH 4 FIGURES)

The name *Fomes geotropus* Cooke has been used commonly in American literature (**23**) for a large fungus that occurs in the southeastern United States. Several authors have recognized the similarity between this fungus and the European species, *F. ulmarius* (Fries) Gill. Recently Lowe (**17**) critically studied these species and he considers the fungus found in this country to be specifically identical with the European species. Cultural studies reported here support this conclusion. The thick context, pinkish color of the pore surface and globose to broadly ovoid spores are the main characters upon which the species is based. It is reported to occur primarily on elm in Europe (**3**). In the southeastern states it has been collected mainly on large old hardwoods (**22**), especially *Magnolia grandiflora* L. (**14**), in which it causes a white soft rot that usually results in a hollowing out of the basal part of the trunks. It has also been collected on bald cypress, *Taxodium distichum* (L.) Rich., by Murrill (**19**) and others, including Long (**15**) who suggested a possible causal relationship with "pecky" cypress. However, recent studies have proven that *Stereum taxodii* Lentz and McKay is the cause of "pecky" cypress (**7, 8**). It also occurs on *Cryptomeria japonica* (L. f.) D. Don in Japan.

The cultural characteristics of *F. ulmarius* are being redescribed here along with those of *Poria ambigua* Bres., with which it has been confused culturally in past work.

CULTURAL CHARACTERISTICS OF RECENT ISOLATIONS

European workers have reported that *Fomes ulmarius* is difficult to obtain from basidiocarps in pure culture (**3**). Numerous attempts made in this country have usually been unsuccessful. However, in November

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TABLE I

CULTURES USED IN THE PRESENT STUDY

No.	Collector	Source	Host	Locality
Fomes ulmarius				
F 40b	K. Aoshima	S*	*Cryptomeria japonica* (L. f.) D. Don	Mie-Ken, Japan
FPRL 241	Dr. Ernest	S	*Ulmus* stump	Horsenden, Bucks, England. Received from J. G. Savory, Princes Risborough, England
L-11696	J. L. Lowe	Sp	*Ulmus* sp.	Latimer, Bucks, England
L-11699	J. L. Lowe	Sp	*Ulmus* sp. stump	Chenies, Bucks, England
L-11701	J. L. Lowe	Sp	*Ulmus* sp.	Chenies, Bucks, England
L-11711	J. L. Lowe	Sp	*Ulmus procera* Salisbury	Tauton, Somerset, England
L-11725	J. L. Lowe	Sp	*Ulmus procera* Salisbury	Nunney, Somerset, England
L-11726	J. L. Lowe	Sp	*Ulmus procera* Salisbury	Little Cheverell, Wiltshire, England
FP 103737	A. S. Rhoads	S	*Magnolia grandiflora* L.	Gulfport (DeSoto Natl. Forest), Miss.
FP 106076	E. R. Toole	R	*Carya aquatica* (Michx. f.) Nutt.	Stoneville (Delta Expt. Forest), Miss.
Poria ambigua				
L-3433	J. L. Lowe	Sp & R	*Acer rubrum* L.	Jamesville, N. Y.
L-3524	J. L. Lowe	Sp	*Acer* sp.	Onondaga Co. (Clark Reservation), N. Y.
L-11673	J. L. Lowe and R. L. Gilbertson	Sp	*Fagus* sp.	Point Rock, N. Y.
FP 55521	L. O. Overholts and F. K. Kaufert	S	*Ulmus* sp.	Ferriday, La.

* S from basidiocarp tissue, Sp from spore print, and R from rot in host wood.

TABLE I—*Continued*

No.	Collector	Source	Host	Locality
FP 55558	L. O. Overholts and F. K. Kaufert	S	*Populus deltoides* Bartr.	Ferriday, La.
FP 71238	J. S. Cooley	S	*Quercus* sp.	Charlottesville, Va.
FP 86349	E. R. Toole	R	*Albizia julibrissin* Durazz.	Tryon, N. C.
FP 86357	M. E. Fowler	R	*Cladrastis* sp.	Washington, D. C.
FP 86439	T. S. Grant	R	*Robinia pseudoacacia* L.	West Point, Miss.
FP 94430	J. A. Stevenson	Sp	*Syringa vulgaris* L.	Washington, D. C.
FP 103063	A. S. Rhoads	Sp	*Carya* sp.	Gainesville (Lake Alice), Fla.
FP 103660	A. S. Rhoads	Sp	*Salix* sp.	Wallaceton (Great Dismal Swamp), Va.
FP 103664	A. S. Rhoads	Sp	Hardwood	Wallaceton (Great Dismal Swamp), Va.
FP 103726	A. S. Rhoads	Sp	*Robinia pseudoacacia* L.	Portsmouth, Va.
FP 103799	A. S. Rhoads	S	*Aralia spinosa* L.	Wallaceton (Great Dismal Swamp), Va.
FP 104025	A. S. Rhoads	Sp	*Melia azedarach* L.	Jacksonville, Fla.
FP 104029	A. S. Rhoads	Sp	Hardwood	Jacksonville, Fla.
FP 104044	H. H. McKay and F. F. Lombard	Sp	Hardwood	Laurel (Patuxent Wildlife Refuge), Md.
FP 104060	G. F. Gravatt	Sp	Buried wood in lawn	College Park, Md.
FP 105032	R. W. Davidson	Sp	*Quercus* sp.	Vicksburg (Bluffs Expt. Forest), Miss.
FP 105254	O. Fidalgo	Sp	*Quercus* sp.	Great Falls, Md.
FP 107020	P. L. Lentz	Sp	*Salix* sp.	Arkansas City, Ark.

1952 A. S. Rhoads made isolations from a basidiocarp collected on magnolia in Mississippi (TABLE I) and sent his culture and specimen to the Beltsville Forest Disease Laboratory for study. A second culture of the same fungus was isolated by E. R. Toole from rot associated with a basidiocarp. These two cultures are considered to be authentic for the species as it occurs in this country and, along with several European isolates from elm and an isolate from Japan, form the basis for the following description of the fungus in culture.

FOMES ULMARIUS (Fries) Gill.

FIG. 1, A–F; FIG. 2, A; FIG. 3, A; FIG. 4

Key Pattern: A-P-M-8-10 and B-P-M-8-10.[3]

Growth Characteristics: Growth medium, forming a mat 22–55 mm in diameter in 7 days; mat white, fine cobwebby to downy, very thin to cleared and appressed around the inoculum, slightly more raised (intermediate) at the margin, homogeneous, adherent at 7 days; mat formed in 14 days 51–90 mm in diameter, white to creamy-white, rarely with small patches of "light buff" color, silky-downy to collapsing cobwebby, narrowly zonate to indistinctly zonate, adherent; margin proper distinct, finely fimbriate; odorless; reverse discoloration slightly yellowish to none; weak oxidase reaction, barely more than a stain, making no growth on gallic and 0 to trace of growth on tannic acid agars in 7 days.

Small patches of fruiting, occasionally formed in old dishes, are "cinnamon-buff," "clay color," "sayal brown," or "russet."

Hyphal Characteristics: All hyphae staining in phloxine (2–)2.3–5 μ in diameter, thin-walled, simple septate, with rare anastomoses, many thin-walled hyphae without contents conspicuous in mounts; vesicular cells present in moderate numbers, terminal, with hyaline, thin walls, broadly ovoid to pear-shaped with the distal end the larger, contents staining uniformly at first, later containing a few scattered or clumped nonstaining small yellowish globules, 9.6–15.6 × 7.2–12 μ; crystals few, odd-angled pieces; basidiospores produced in old cultures, hyaline, globose to broadly ovoid, 4.5–7 × 4.5–5.5 μ.

[3] The methods employed in studying the cultures and the arrangement of the descriptions and explanation of the key pattern have been published previously (**6**). Some of the methods and terminology also relate to the studies by Campbell (**2**), Long and Harsch (**16**), and Nobles (**20**). Mat descriptions and growth rates excepting constant temperature studies were based on 7- and 14-day-old Petri dish cultures incubated at 25° C on 2½% Fleischmann's diamalt agar. Test-tube cultures were grown at room temperature (about 25°) in diffused light. The test for oxidase reaction follows the method described by Davidson et al. (**5**). Colors in quotation marks are according to Ridgway (**25**).

Temperature Relations: Optimum 28°, killing at 38–40° C (TABLE II).

Test-tube Cultures: In 28 days mat creamy-white to "cartridge buff" or "pinkish buff," frequently with patches of "light ochraceous-buff," "ochraceous-buff," and "ochraceous-salmon" around the inoculum, subfelty, covered with sparse downy aerial growth, narrowly zonate, occa-

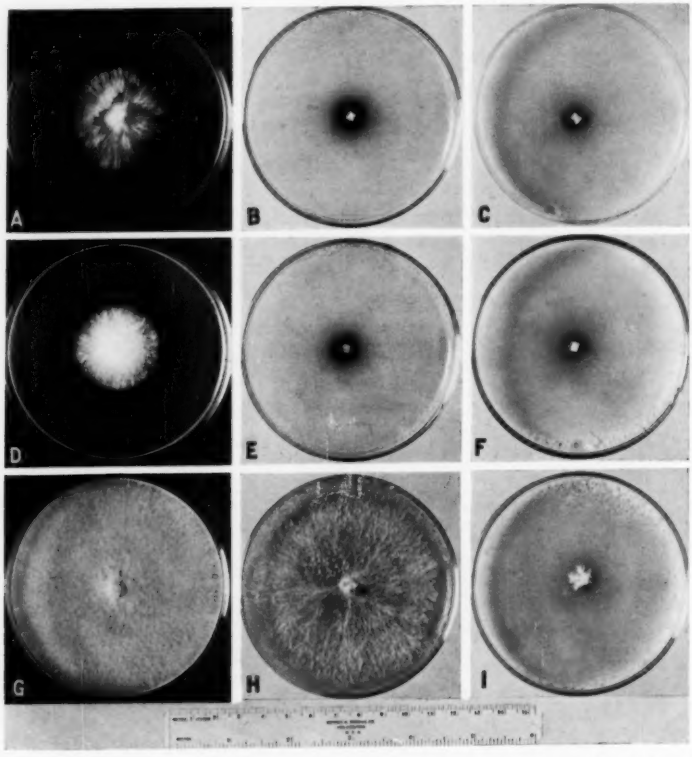

FIG. 1. One-week-old cultures on malt agar and gallic and tannic acid agars. A, D, and G, malt; B, E, and H, gallic acid; C, F, and I, tannic acid. A to C, *Fomes ulmarius*, FPRL 241; D to F, *Fomes ulmarius*, FP 103737-S; G to I, *Poria ambigua*.

sionally with raised coalescing creamy-white to "clay color" small mounds or definite small merulioid to finely poroid fruiting patches of "pinkish cinnamon" on the slant; mat on agar cylinder "pinkish buff"

becoming nearly white in the marginal growth at base of cylinder, downy in thick and thin patches, zonate; very slight if any reverse discoloration.

Type of Decay: In American literature *F. ulmarius* (as *F. geotropus*) is reported to cause a soft white rot of hardwoods (**1**) and to occur on dead wood or wounds in living trees (**14, 23, 24**) (FIG. 4, B). In England it is reported as causing a brown butt rot of elm (**3**).

TABLE II

RATES OF GROWTH ON MALT AGAR AT CONSTANT TEMPERATURES

	14° C	18°	22°	26°	28°	30°	32°	34°	36°	38°	40°
Fomes ulmarius 3 isolates 7 days	trace	22.89*	29.33	38.00	48.66	38.44	25.11	13.77	0	0	0
Poria ambigua 27 isolates 3 days	trace to 14.47	29.25	46.51	59.94	70.07	75.31	73.32	69.56	0 to 63.00	0 to 38.00	0 to trace

* Diameter measurements are in millimeters, averages of growth in triplicate in Petri dishes.

Cultures Studied: See TABLE I.

Remarks: The distinguishing cultural characteristics of this species are its white and more or less fragile mat, weak oxidase reaction, lack of clamps, and presence of vesicles.

Difficulty in isolating from sporophores of this fungus has been mentioned. This also was the experience with the recently collected sporophores in England but spores deposited directly on culture medium and retransferred to fresh tubes of sterile medium produced uncontaminated cultures after an incubation period of 5 to 10 days. Also, cultures were obtained from sporophore tissue by using older malt agar medium having a dry surface on which bacteria did not develop rapidly. By careful examination of cultured portions from interior context and old pore layers pure cultures were obtained from 3 of 4 specimens.

Cartwright and Findlay (**3**, p. 111) have reported the occurrence of a few thick-walled chlamydospores in the decayed wood and a "few oval chlamydospores (about 10 μ diam)" in the cultures. We have been unable to verify the presence of chlamydospores in the rot of the American specimens and, on the basis of the thin walls of the spherical bodies as produced in the cultures, are inclined to consider them to be vesicles rather than poorly developed chlamydospores.

According to information recently received from the Forest Products Research Laboratory, Princes Risborough, England, the culture of "*Fomes geotropus*" (FPRL 221) used in the comparison study with *Fomes ulmarius* (FPRL 241) at that Laboratory was the Overholts'

isolate obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland. This isolate is known now to be *Poria ambigua*. Cartwright's and Findley's interpretation of *F. geotropus* was based on this misidentified Overholts' culture, which explains their conclusion that the two species are not identical (3).

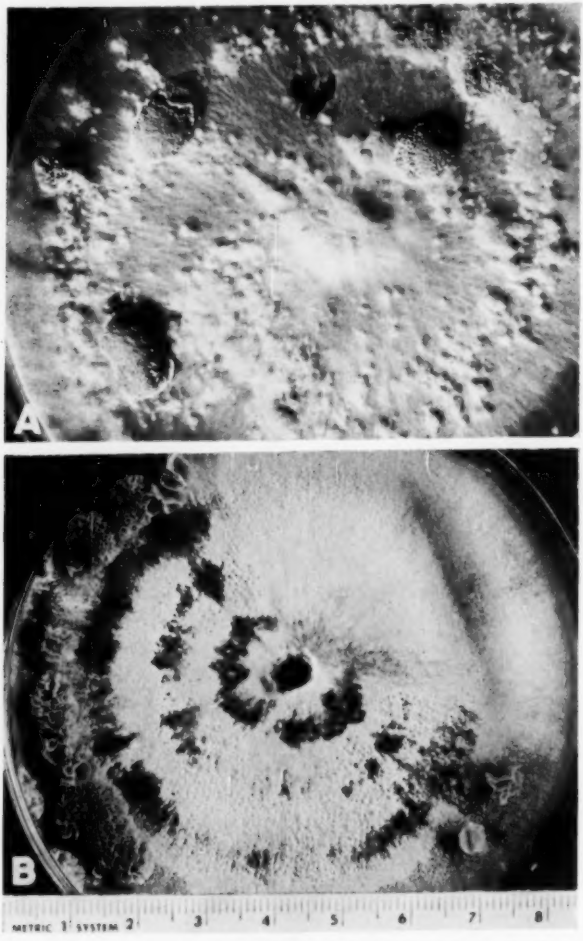

FIG. 2. A, *Fomes ulmarius* (FP 103737-S) fruiting in 3½-week-old Petri dish. B, *Poria ambigua* (rot culture from living tree) showing characteristic poroid to foliose or funnel-shaped fruiting in 2-week-old Petri dish.

OLDER CULTURES DESIGNATED AS FOMES GEOTROPUS

The first published description of cultures of *"F. geotropus"* was by Campbell (**2**) in 1938, based on two isolates from basidiocarps collected by L. O. Overholts and F. K. Kaufert, numbers FP 55521-S and FP 55558-S. It was redescribed by Davidson et al. (**6**) in 1942, based on 29 decay isolations from sapwood and heartwood in oaks, determined by comparison with the two Overholts' isolates. These 29 rot isolates were mainly from Hepting's (**12**) study of decay following fire in young Mississippi Delta hardwoods. Some of these isolates were also used as *"F. geotropus"* in the oxidase reaction study published in 1938 (**5**).

Recent study of the two Overholts' isolates and the subsequently isolated cultures has shown that, in our opinion, there is no question but that they are *Poria ambigua*. The probable mix-up of the Overholts' isolates occurred prior to Campbell's study of them. The following description of *P. ambigua* is based on isolates from basidiocarps identified by J. L. Lowe and R. L. Gilbertson, as well as the older cultures available for study. The description given here for *P. ambigua* is practically the same as that previously given for *F. geotropus* in the publications cited above.

PORIA AMBIGUA Bres. FIG. 1, G–I; FIG. 2, B; FIG. 3, B

Key Pattern: A-P-F-9-11-14-16, B-P-F-5-6-9-11-14-16, A-P-F-5-6-9-11-14-16.

Growth Characteristics: Growth rapid, making a mat over 90 mm in diameter in 5 to 6 days; in 14 days mat white, more or less thin felty, adherent, somewhat tough, azonate, occasionally thinner and cleared around the inoculum, surface smooth-appressed or frequently floccose, downy, or pitted-nodulose especially beyond the central zone, often with raised coarsely foliose to irregularly poroid or funnel-shaped basidiocarp tissue of creamy white color ("pale olive-buff," "cartridge buff," "tilleul-buff," occasionally to "cream color") developing as large continuous masses against the sides of the Petri dish or scattered on the mat surface or occasionally as small isolated rosettelike groups; odor none to slight, nondescript or occasionally mushroom; margin proper distinct, finely fimbriate; no reverse agar discoloration; weak oxidase reaction, frequently spotted and fading on gallic and occasionally hardly more than a stain on tannic, making mats (21–)45–90+ mm diam on gallic and trace to 35(–55) mm diam on tannic acid agars in 7 days.

Hyphal Characteristics: Hyphae staining in phloxine, 1.5–10 μ in diameter, septate, without clamps, often incrusted with numerous small octohedral crystals, the larger hyphae (5–10 μ) staining heavily, broken into free-floating 1- to 5-celled segments, individual cells 70–137 μ in

length and occasionally branching, very conspicuous in the mounts; staghorn hyphae abundant in most isolates, staining, 1.2–1.7 μ diameter, with short blunt branches; nonstaining hyphae, 1.7–2.3 μ diameter, sparingly septate, occasionally branching at right angles, lacking contents but with hyaline, refractive walls that are only slightly thickened, rarely containing small spaces of completely thickened walls; mature basidia occasionally present at 14 days, basidiospores hyaline, smooth, oblong-ellipsoid to ellipsoid, 3.5–6 × 2.5–3.7 μ; crystals medium to small octohedrals.

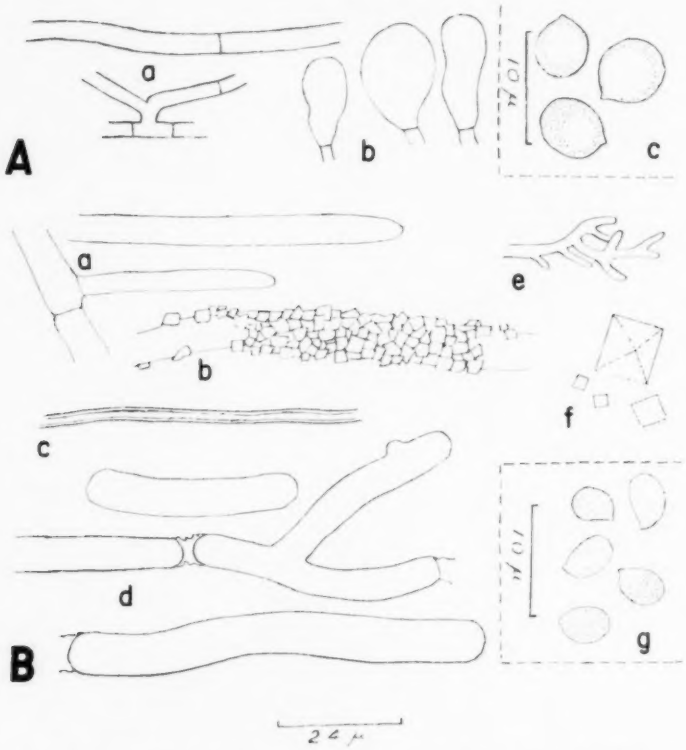

FIG. 3. A, *Fomes ulmarius:* a, hyphae, b, vesicular cells, c, basidiospores; B, *Poria ambigua:* a, hyphae from marginal growth, b, incrusted hypha, c, nonstaining hypha, d, hyphal segments, e, staghorn hypha, f, crystals, g, basidiospores.

Temperature Relations: Optimum 30–32°, killing at 46° C (TABLE II). Growth was extremely variable at the higher temperatures. Cer-

tain isolates grew from 36°–42°, while others made no growth. 42% were killed at 44°.

Test-tube Cultures: In 28 days mat on slant and agar cylinder white, thin, felty, usually without aerial mycelium but occasionally slightly floccose on lower part of cylinder; usually with thin lamellate to poroid fruiting areas "cream-buff," "cartridge buff," "light grayish olive," or "deep olive-buff," formed on tops or sides of slants and occasionally between glass and agar cylinder.

Type of Decay: An examination of numerous wood specimens on which *Poria ambigua* was fruiting suggests that it should be a white rot, as has been reported (**6, 12**). In any case, none of the specimens show any indication that a typical brown cubical rot is present. The oxidase reaction is weak as is the case with the other species described in this paper; however, in view of the fact that there is often a weak reaction and in the absence of brown cubical rot in association with the basidiocarp, it is considered a white rot species.

Cultures Studied: In addition to the isolates listed in TABLE I there were also included in this study nine cultures isolated from rot in Mississippi Delta hardwoods by E. R. Toole, eight of the Hepting rot isolates from oak in Louisiana (**12**), and four miscellaneous rot isolates from California, Oklahoma, and Virginia.

Remarks: The distinguishing cultural characteristics of this fungus are its rapid growth on malt agar; absence of clamps; the presence of staghorn hyphae, heavily staining large hyphal segments, and incrusted hyphae; and, when present, the large coarse foliose masses of basidiocarp tissue.

The above "key pattern" and description differ somewhat from those of the previously published descriptions for *"Fomes geotropus."* The diagnostic value of the large hyphal segments is considered to be sufficient to warrant being included in the key pattern as "special structures" (No. 16). Large hyphae are typically found in basidiocarps of *P. ambigua* (**18**).

During the course of years the Overholts' cultures, FP 55521-S and FP 55558-S, have been distributed as *Fomes geotropus* to a number of research workers at other laboratories by the Forest Disease Research Laboratory of the U. S. Department of Agriculture at Beltsville (formerly the Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering) and by others also having these cultures. The present authors would like to make published correction of these misidentifications wherever possible. A number of publications have

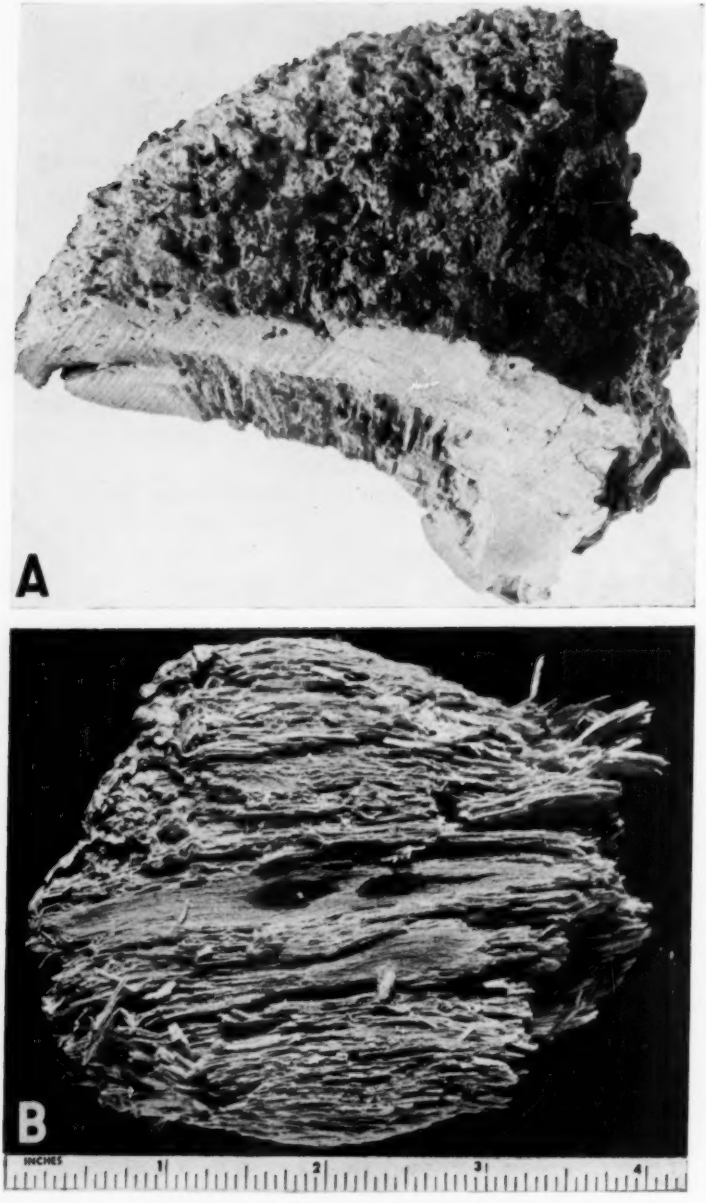

FIG. 4, A–B.

appeared in which various scientific information based on these isolates as *F. geotropus* has been reported. Renumbering isolates without citing the original collectors' numbers, an unfortunate practice followed by some laboratories, makes it impossible to positively identify all of the publications reporting data based on these old isolates. However, the following can be so identified, either by the authors' published identification of the cultures by their original numbers or from shipment records here at this Laboratory: Day et al. (**9**) as culture No. 9, Jennison et al. (**13**), and Robbins et al. (**26**) used FP 55521-S as *F. geotropus* in their studies.

The 1957 "List of Cultures" of the Centraalbureau voor Schimmelcultures at Baarn, Holland, (**4**) gives Overholts as the source of their isolate of *F. geotropus*. This culture was examined recently and was found to be *P. ambigua*. The Forest Products Research Laboratory at Princes Risborough, England, received their *F. geotropus* isolate (FPRL 221) from CPS, "isolated by Overholts." Culture No. LCF 612, listed as *Fomes geotropus*, in the catalog of the Division de Fitopatologia, Buenos Aires, Argentina (**30**), is FP 55521-S, *P. ambigua*.

Cultural identifications made at this Laboratory by comparison studies using the Overholts' isolates have resulted in other misidentified cultures. In Toole's recent publication (**27**), *F. geotropus* associated with fire scars as listed in TABLE 2 under "Red Oaks" should be *"Poria ambigua."* Also, the isolate used as *F. geotropus* in Toole's inoculation studies (**28**) reported in 1956 was *P. ambigua*.

DISCUSSION

The original description of cultures of *F. ulmarius* (*F. geotropus*) was based on two Overholts' cultures at least one of which was supposed to be a basidiocarp culture. The new description is also based on two cultures of the American fungus, only one of which is a basidiocarp isolation. The Overholts' cultures have the same characteristics as those of *Poria ambigua* obtained from spores. Fruiting in isolates of *F. ulmarius* agrees with the American concept of this species. These two facts lead the authors to believe that the cultures of the two species are now correctly identified. This identification is further strengthened by the similarity of cultures to those of *F. ulmarius* from English elm.

FIG. 4. *Fomes ulmarius*. A, Basidiocarp (FP 103737) collected on *Magnolia grandiflora* in Mississippi by A. S. Rhoads in 1952. B, Stringy white rot in *Nyssa aquatica* associated with basidiocarp (FP 12379) collected in Louisiana by W. H. Long in 1913.

There seems to be no definite report of *F. ulmarius* causing a brown rot in host tissue on which it occurs in this country. The fact that Long (**15**) thought it to be the probable cause of "pecky" cypress might indicate that it is a brown rot species, but even "peckiness" in cypress (**8, 29**) is not a very clear-cut brown rot, especially in early stages of development. Boyce (**1**) refers to this fungus as causing "a soft white rot of hardwoods" and this is our opinion. The wood is reduced to a soft white condition rather quickly or at least there is usually no large amount of intermediate decay. An examination of a considerable number of herbarium specimens, collected mainly by Long (**15**), indicates that the white decay consisted of wet stringy to soft woody tissue, which collapsed and dried to a parchmentlike mass. This habit of rapid disintegration apparently accounts for the hollow condition associated with infections. No specimens seen have indicated the presence of a brown carbonizing rot. Both the American and European isolates give weak oxidase reactions on gallic and tannic acid agars. They also give inconclusive results with alcoholic gum guaiac solution prepared according to Nobles' formula (**21**).

Hemmi (**10**) and Hemmi et al. (**11**) illustrate and describe a rot in *Cryptomeria japonica* as being similar to that of "pecky" cypress. This does not seem consistent with conditions found here even though Long (**15**) did indicate observing pecky decay in several cypress trees on which basidiocarps of *F. ulmarius* were collected. Also, the temperature response of cultures of the Japanese fungus studied by Hemmi do not correspond to those of cultures studied here. Although we are at present uncertain regarding the type of decay associated with this fungus in *Cryptomeria,* the culture recently received from K. Aoshima is *F. ulmarius* as described in this paper.

SUMMARY

A recent culture from a basidiocarp of *Fomes ulmarius* indicates that cultures previously used to describe the species in this country were incorrectly identified. The older cultures, which had been so designated, were actually cultures of *Poria ambigua.* The cultural characteristics of these species are described.

The two species are weak reactors when grown on gallic or tannic acid media but evidence from decay associated with the basidiocarps indicates that they cause white rots in hardwoods. *Fomes ulmarius* occurs on living trees, mainly hardwoods, in the southeastern United States and on hardwoods, especially elm, in European countries. Decay isola-

tion studies also indicate that *P. ambigua* commonly occurs on hardwoods in the southeastern United States and is prevalent in decay associated with fire wounds in some areas of the South.

ACKNOWLEDGMENTS

The authors are indebted to R. L. Gilbertson for cultures and for examination and identification of specimens and to Kiyowo Aoshima, A. S. Rhoads, J. G. Savory, E. R. Toole, and others for supplying cultures.

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MORPHOLOGICAL DEVELOPMENT AND NUCLEAR BEHAVIOR IN THE GENUS TAPHRINA [1]

C. L. KRAMER [2]

(WITH 122 FIGURES)

The genus *Taphrina* as now defined (**11**) has been a favorite research subject for many workers. Yet many details in the development of the species have not been completely understood or have been a subject of disagreement. The majority of the comprehensive cytological and morphological studies such as those of Eftimiu (**1**), Fitzpatrick (**2**), Mix (**9**), and Martin (**8**) has been of *T. deformans*. The present work includes studies of species considered to represent some of the major variations in morphological types that occur within the genus. These are listed as follows: *Taphrina carveri* Jenkins, representing species with subcuticular mycelium and asci with stalk cells; *T. ulmi* (Fkl.) Johanson with subcuticular mycelium which does not fragment into individual ascogenous cells, and asci with stalk cells; *T. virginica* Sadebeck with subcuticular mycelium and asci which lack stalk cells; *T. populi-salicis* Mix with subcuticular mycelium, asci with stalk cells and precocious ascospore development; *T. deformans* (Berk.) Tulasne with intercellular and subcuticular mycelium and asci with stalk cells. This paper attempts to clarify and expand knowledge of the types of nuclear behavior and morphological development that exist within the genus. Although only 5 species are discussed, many of the observations have been substantiated by studies of numerous other species.

MATERIALS AND METHODS

The cytological studies were made primarily from materials killed and fixed in formalin-propiono-alcohol as proposed by Johansen (**6**). Portions of the fixed material were embedded in paraffin and cut on a

[1] Contribution No. 562, Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station, Manhattan, Kansas. Department Serial No. 722.

[2] The author wishes to thank Dr. N. M. McClung and the late Dr. A. J. Mix for their interest and helpful suggestions in the undertaking of this problem.

rotary microtome from 3 to 15 μ thick. Of the several stain techniques used, Heidenhain's iron hematoxylin was found most satisfactory.

Many of the morphological studies were made from free hand sections of dried and fresh material stained with acid fuchsin or fast green. However, best results for studying the somatic mycelium and the development of the hymenium were obtained by stripping a layer of epidermis from leaves with a microscalpel. The stripped epidermis was then mounted in glycerin containing acid fuchsin or fast green. Various bleaches and clearing agents were used with only moderate success.

Drawings were made with a camera lucida at a magnification of $\times$ 1860. Observations were made using a Leitz compound microscope with a 2 mm 1.30 apochromatic oil immersion objective and a 15 $\times$ compensating ocular.

Collections used in this study are: *Taphrina carveri* Jenkins on *Acer saccharinum* L., Lutesville, Missouri, May 30, 1941, *A. J. Mix* T-881. Several years later Dr. Mix returned to this location and on the same tree from which the earlier collection was made, found only a few immature lesions. In June, 1955, the author also visited this location, but it was learned that the fungus had not appeared for 6 or 7 years. This species is quite rare and is known from only 3 other locations (**4, 5**). *Taphrina virginica* Sadebeck on *Ostrya virginica* Willd.: Baldwin, Kansas, *AJM*, TK-221; Baldwin, Kansas, *C. L. Kramer* TK-84. *Taphrina ulmi* (Fkl.) Johanson on *Ulmus rubra* Muhl.: Leavenworth, Kansas, *CLK* TK-89; Leavenworth, Kansas, CLK TK-227; on *U. americana* L.: Ottawa, Kansas, *CLK* TK-79; Lawrence, Kansas, *CLK* TK-255; on *U. alata* Michx.: Clinton, Kentucky *CLK* TK-237; on *U. crassifolia* Nutt.: Mooringsport, Louisiana, *R. L. McGregor* T-1252. This is the first record of this species on *U. crassifolia*. *Taphrina deformans* (Berk.) Tulasne on *Prunus amygdalus Batsch.* (almond): Aberdeen, Scotland, 1939, *AJM* (no herbarium specimen of this collection was preserved); on *Prunus persica* L.: Ottawa, Kansas, *CLK* TK-80; Leavenworth, Kansas, *CLK* TK-90. *Taphrina populi-salicis* Mix on *Populus trichocarpa* Torr. & Gray: Palo Alto, California, *R. H. Thompson* (TYPE collection); Aptos, California, *H. E. Thompson & C. E. Scott*, August 11, 1954; Monterey County, California, *K. L. & A. J. Mix*, August 6, 1954; on *Populus fremontii* S. Wats., Berkeley, California, *H. N. Hansen*, July 10, 1938; Sacramento, California, *H. E. Parks & W. S. Fields*, June 8, 1924. Herbarium specimens of the collections cited above are in the mycological herbarium of Kansas University, Lawrence.

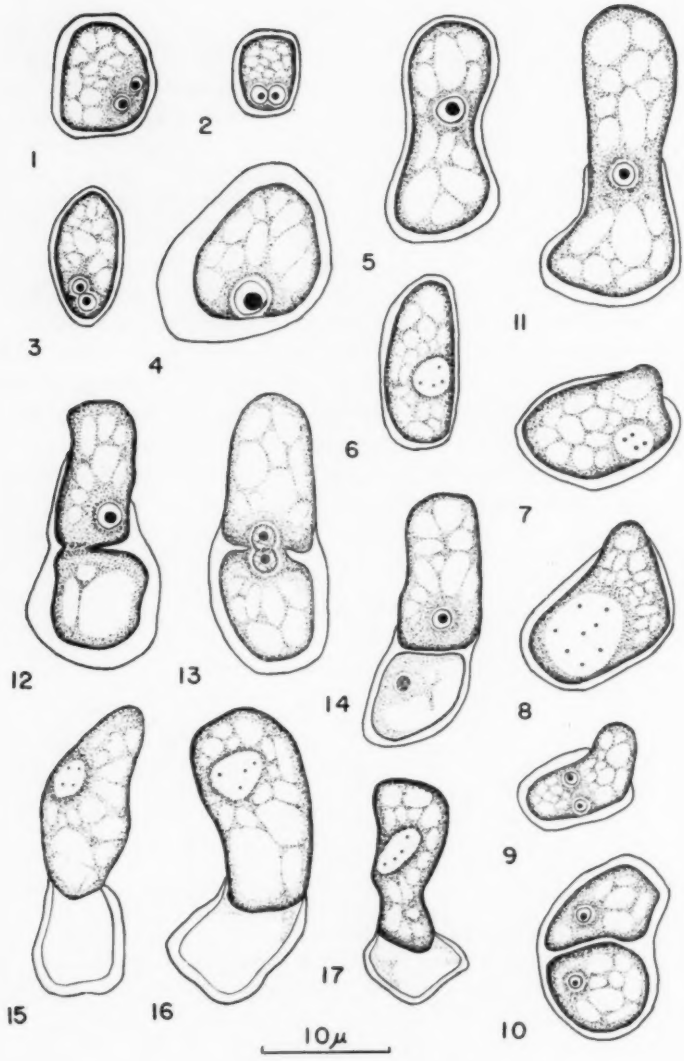
OBSERVATIONS

Taphrina carveri Jenkins. The preserved material available was in the mature ascus stage, and young stages were scarce. However, very small ascogenous cells were found to be dicaryotic (FIGS. 1–3), indicating the probability of dicaryotic mycelium as has been found in all other species studied.

The subcuticular mycelium segments into short cells that develop to the typical thick-walled, irregularly-shaped ascogenous cells. These are at first dicaryotic, with karyogamy occurring to form a rather large diploid fusion nucleus (FIG. 4). The ascus arises by the splitting of the outer ascogenous cell wall which allows the inner membrane to emerge and form a papilla (FIGS. 11–14). In some of the other species which have stalk cells, such as *T. deformans*, the ascogenous cell wall does not rupture, but instead stretches to allow the elongation of the papilla to form the ascus.

During the development of the young ascus, the fusion nucleus undergoes a division (FIGS. 6–9, 13), which may begin before the ascogenous cell wall has ruptured (FIG. 6), or it may occur during the emergence of the papilla (FIGS. 7–9). In one instance the nuclear division was completed and the septum, which cuts off the stalk cell, was formed before the ascogenous cell wall had ruptured (FIG. 10). One of the nuclei was located in what would become the ascus, while the other remained in the location of the stalk cell. This is an equational division with four chromosomes evident in the early division phases (FIGS. 6, 7) and four moving to each pole at anaphase (FIG. 8) indicating four chromosomes in the diploid stage and two in the haploid. One of the daughter nuclei migrates into the distal portion of the developing ascus while the other remains or moves into the region of the stalk cell and soon disintegrates. This, therefore, is similar to the nuclear behavior reported by Martin (**8**) for *T. deformans*.

The septum which cuts off the stalk cell in this species is formed in the manner usually encountered in fungi. The wall progresses inward with annular thickenings until it has cut through the cytoplasm (FIGS. 12, 13). Following the formation, the nucleus of the ascus undergoes meiosis followed by a third division which is equational, giving rise to the haploid ascospore nuclei. In division I of meiosis, four spherical chromosomes, which apparently represent pairs of chromatids, and a large nucleolus are evident in early division phases (FIGS. 15–17). At anaphase, four chromatids have moved to each pole (FIGS. 18, 19).



FIGS. 1–17.

FIGS. 1–17. *Taphrina carveri* Jenkins. × 1860. FIGS. 1–3. Ascogenous cell containing conjugate pair of nuclei. FIG. 4. Ascogenous cell with large fusion nucleus. FIG. 5. Elongating ascogenous cell with large fusion nucleus. FIG. 6.

Early phases of division II could not be distinguished from late phases of division I. In FIGURE 20, one of the resulting nuclei is in interphase while the other is in either a late phase of division I or in an early phase of division II. At anaphase of division II, two chromatids are evident at each pole (FIG. 21). These divisions result in the formation of four haploid nuclei (FIG. 22).

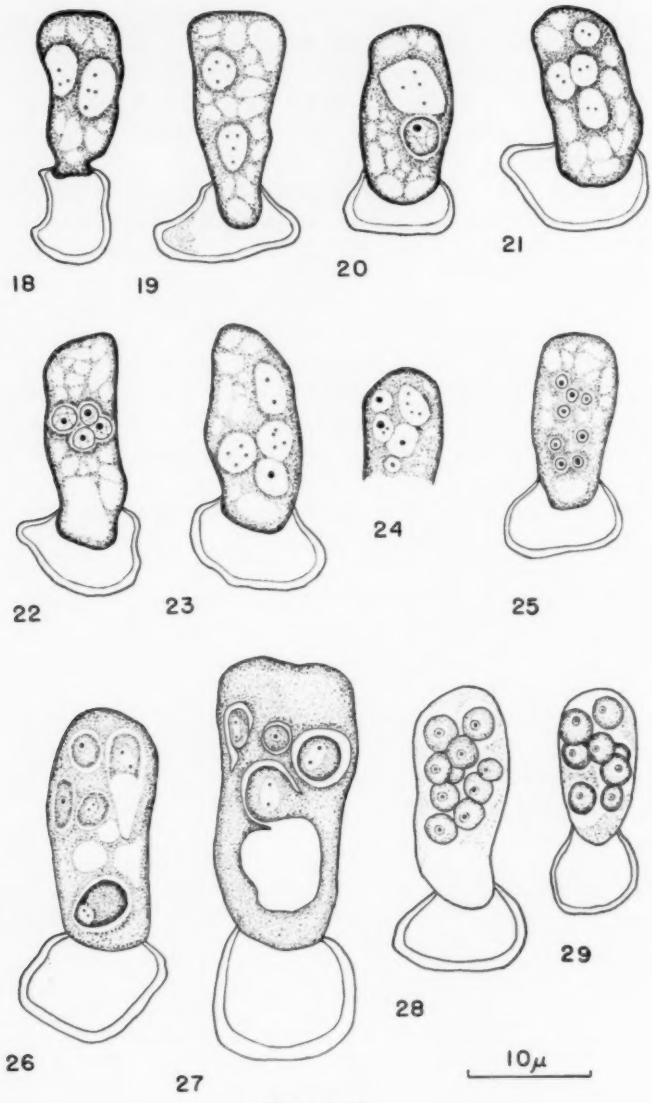
Due to the minuteness of the chromosomes, it was often impossible to distinguish between late phases of one division and early phases of the next. This might be said of FIGS. 18–21 and in similar situations with the species discussed subsequently.

The eight haploid ascospore nuclei (FIG. 25) are formed as a result of a third equational division of the four haploid nuclei. Apparently this last division may not occur simultaneously in all four nuclei. Some of the nuclei may be in early division phases with two chromosomes evident, while others, nearing the end of division, may show four chromatids. At the same time there may be other nuclei in interphase (FIGS. 23, 24).

Frequently large portions of cytoplasm containing one or more nuclei are cut out by coalescence of vacuoles (FIGS. 26, 27). The cytoplasm containing the eight ascospore nuclei forms a layer next to the ascus wall. The spores apparently are delimited by the fusion of vacuoles in the same manner as the large portions of cytoplasm which are separated earlier. A small amount of epiplasm remains in the ascus after spore formation (FIGS. 28, 29).

Budding of the ascospores, while still within the ascus, occurs commonly in the material studied. However, there are rarely more than 16 to 18 blastospores per ascus; considerably fewer than is usually found in species such as *T. virginica*.

Early division phase of fusion nucleus undergoing mitotic division; 4 chromosomes evident. FIG. 7. Ascogenous cell wall rupturing to allow emergence of ascus during mitotic division of fusion nucleus. FIG. 8. Later phase of same mitotic division showing 8 chromatids. FIG. 9. Emerging ascus; 2 nuclei interpreted as resulting from equational division of fusion nucleus. FIG. 10. Equational division of fusion nucleus and formation of stalk cell completed before emergence of ascus from ascogenous cell. FIG. 11. Emergence of ascus before division of fusion nucleus. FIG. 12. Formation of stalk cell by constriction of plasma membrane. FIG. 13. Formation of stalk cell; one of the nuclei resulting from equational division entering stalk cell, while the other is entering ascus. FIG. 14. Young ascus with stalk cell containing diploid nucleus undergoing degeneration. FIGS. 15–17. First meiotic division in young ascus; 4 chromosomes evident in early division phase.



FIGS. 18–29.

FIGS. 18–29. *Taphrina carveri* Jenkins. × 1860. FIGS. 18, 19. Interpreted either as late phase of first meiotic division or early phase of second meiotic division.

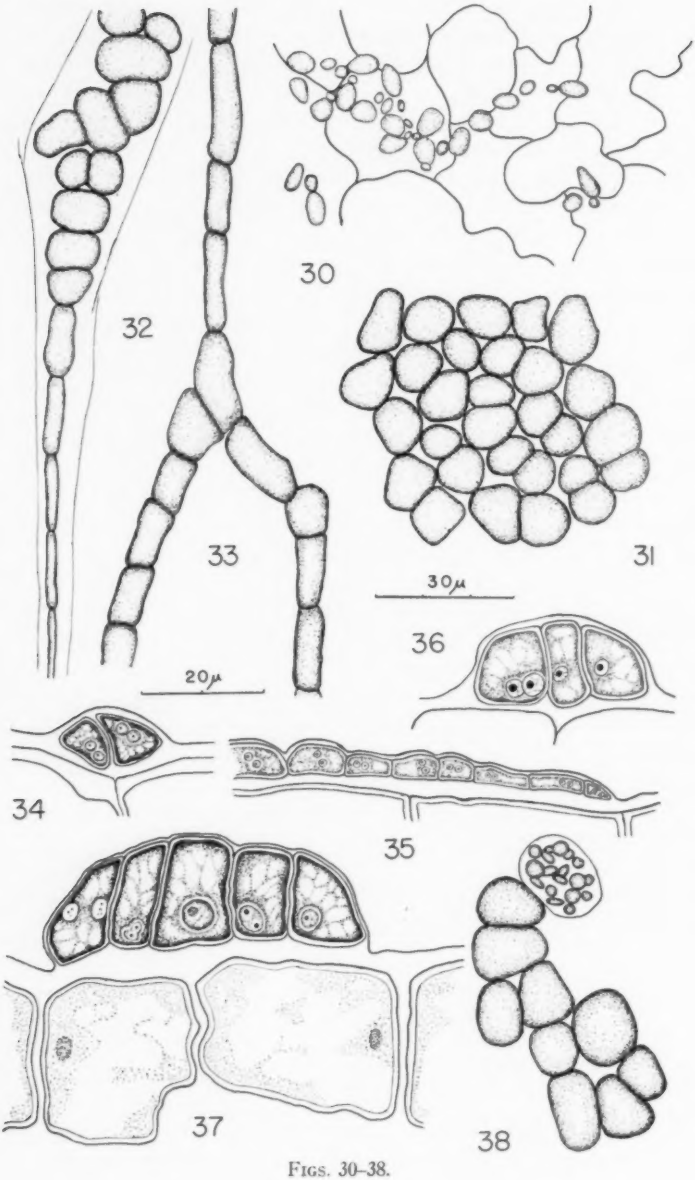
Taphrina virginica Sadebeck. In most species of *Taphrina,* the ascogenous layer is formed by the fragmentation of the vegetative mycelium into ascogenous cells. In this species, however, the vegetative phase consists of yeastlike cells which bud repeatedly to form colonies beneath the cuticle (FIG. 30). These cells enlarge to form a compact hymenium of ascogenous cells (FIG. 31). Hyphae develop only occasionally, and those that do form are considered as distributive hyphae for they often connect two colonies of ascogenous cells. FIG. 32 illustrates one such hypha arising from a colony of bud cells. The other end of this particular hypha (not figured) also terminated in a colony of ascogenous cells. Occasionally such hyphae branch (FIG. 33), but they do not form extensive mycelium such as is produced by some of the other species of *Taphrina.* As these hyphae mature, they enlarge and become separated into individual cells. The cells apparently remain capable of division for some time, but eventually produce a layer of ascogenous cells. Colonies of ascogenous cells and young asci in various stages of development may be seen in FIGS. 34–38.

The cytoplasm is very dense in the young cells but becomes somewhat vacuolated as they increase in size. A binucleate condition exists in all stages of this vegetative phase (FIGS. 34–36, 39).

In *T. virginica,* the ascus is formed by the extension of the ascogenous cell wall. Fusion of the two haploid nuclei (FIG. 40) producing a diploid nucleus (FIG. 41) occurs during the elongation of this cell, the young ascus. Since an equational division preceding meiosis has been reported only in species with stalk cells (**3, 8**), an important question is posed regarding the existence of this mitotic division in those species which lack stalk cells. Such a mitotic division has not been found to occur in this species which lacks a stalk cell.

Division I of meiosis of the fusion nucleus occurs with four chromosomes appearing in early division phases (FIGS. 42–44) and four chromatids which move to each pole in anaphase (FIGS. 45, 46). The ascus illustrated in FIG. 42 shows one centromere, while two centromeres are evident in the ascus of FIG. 46.

FIG. 20. One nucleus in interphase while other is beginning second meiotic division. FIG. 21. Interpreted either as late phase of second meiotic division or early phase of third mitotic division; reduction to haploid number of chromosomes is complete with each of four nuclei containing two chromosomes. FIG. 22. Four haploid nucleate ascus. FIGS. 23, 24. Mitosis of 4 haploid nuclei. FIG. 25. Eight nucleate ascus. FIGS. 26, 27. Cytoplasm of ascus being delimited into smaller portions, often occurring before ascospore nuclei are formed. FIGS. 28, 29. Mature asci with 8 ascospores or with slight production of blastospores.



FIGS. 30–38.

In many of the division figures, there appeared to be a slight difference in the size of the chromosomes, one pair being a little larger than the other (FIGS. 42, 43, 45, 48). However, this difference in size was not discernible in all division phases, consequently its existence could not be definitely established.

In division II of meiosis, four chromosomes are evident in the early stages of division (FIG. 48), and two chromosomes move to each pole at anaphase (FIG. 49) giving rise to the four haploid nuclei (FIG. 50). The chromosome count is the same as in the species discussed previously; four in the diploid and two in the haploid condition.

A third equational division occurs in the usual manner giving rise to the eight ascospore nuclei (FIG. 51). The large portions of cytoplasm containing one or more nuclei, as described in the preceding species, were not seen in *T. virginica*. However, the spores seem to be formed in the same manner as described above.

Budding of the ascospores within the ascus is a common occurrence in this species. Mature asci are often filled with many small blastospores (FIGS. 52, 53).

Taphrina ulmi (Fkl.) Johanson. The young mycelium of *T. ulmi* is composed of fine, freely branching subcuticular hyphae (FIGS. 55–58). The septa, which are quite thick and highly refractive, were often called "plate septa" by earlier workers. The cells at the tips of the advancing hyphae, near the margins of the lesions, are usually irregular in outline with short developing branches (FIG. 58). The cytoplasm of the young mycelium is very fine textured and of uniform consistency. All cells of the mycelium are dicaryotic, containing a single pair of nuclei (FIG. 60). As the mycelium matures, the cells increase in size and are quite irregular in shape (FIG. 59).

The mycelium of this species does not separate into the individual ascogenous cells as it does in other species of *Taphrina*, but remains continuous. The asci arise from the cells of the mycelium; however,

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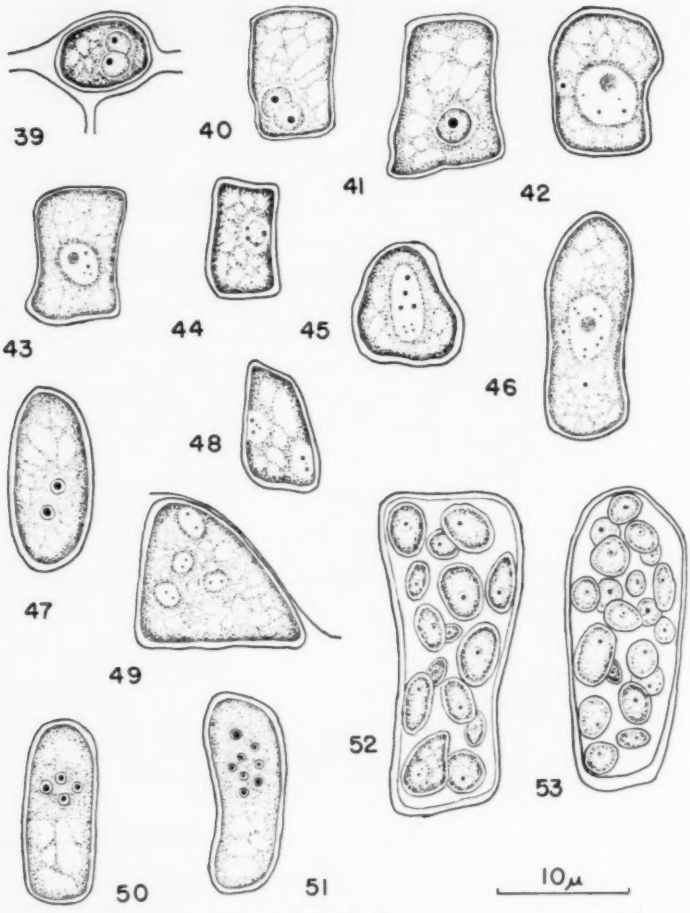
FIGS. 30–38. *Taphrina virginica* Sadebeck. FIGS. 30–33, 38, × 1395 (use 30 μ scale). FIGS. 34–37, × 930 (use 20 μ scale). FIG. 30. Early stage of infection; subcuticular, budding yeastlike cells. FIG. 31. Early stage in developing ascogenous layer, ascogenous cells formed from yeastlike cells. FIG. 32. Distributive hyphae effecting spread of fungus over leaf surface. FIG. 33. Distributive hyphae developing into ascogenous cells. FIG. 34. Young ascogeneous cells in cross section. FIGS. 35, 36. Cross section of young ascogenous layer. FIG. 37. Cross section of ascogenous layer with cells in various stages of nuclear division. FIG. 38. Mature ascogenous layer as it appears on a portion of stripped host epidermis.



FIGS. 39–53.

FIGS. 39–53. *Taphrina virginica* Sadebeck. × 1860. FIG. 39. Ascogenous cell with pair of conjugate nuclei. FIG. 40. Fusion of conjugate nuclei. FIG. 41. Ascogenous cell with large fusion nucleus. FIGS. 42–44. Early division phase of first meiotic division showing 4 chromosomes. FIG. 45. Late phase of first meiotic division showing 8 chromatids. FIG. 46. Ascogenous cell elongating into ascus with 2 centromeres present, nucleolus and 8 chromatids evident in dividing nucleus. FIG. 47. Two nucleate ascus. FIG. 48. Interpreted as late phase of first meiotic division or early phase of second meiotic division. FIG. 49. Interpreted as late phase of second meiotic division or early phase of third equational division. FIG. 50. Four nucleate ascus. FIG. 51. Eight nucleate ascus. FIGS. 52, 53. Mature ascus with numerous blastospore formation.

not all cells give rise to asci (FIG. 59). Fusion of the dicaryon occurs in the ascogenous cells (FIGS. 61–63).

After nuclear fusion, the ascogenous cell wall ruptures allowing the inner membrane to emerge as a papilla which becomes the young ascus (FIGS. 61–63). The fusion nucleus usually migrates into the papilla where it undergoes an equational division. Four chromosomes are evident in the early stages of division (FIG. 64), and four chromatids move to each pole at anaphase (FIG. 65). This indicates a chromosome number of four in the diploid and two in the haploid stage.

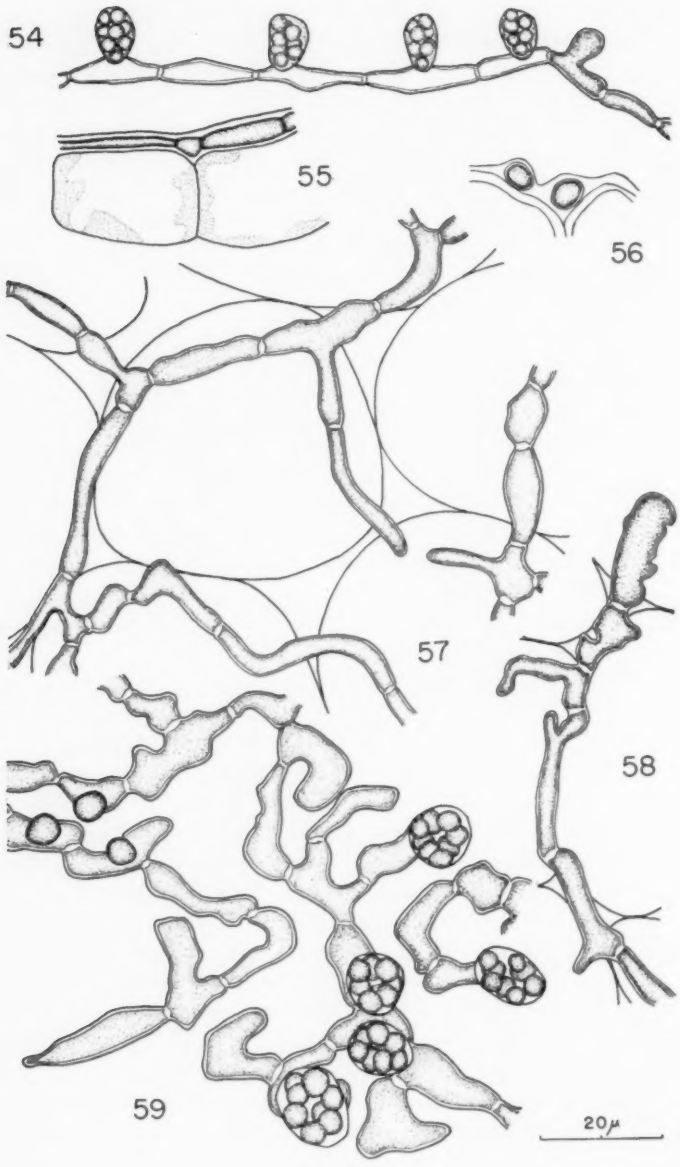
As in *T. carveri,* one of the nuclei resulting from this division migrates into the stalk cell while the other remains in the ascus (FIG. 66). After the formation of a septum which cuts off the stalk cell, the nucleus and protoplasm disintegrate leaving it empty.

Reduction of the diploid nucleus occurs in the young ascus after formation of the septum. The nuclear behavior is the same as in *T. carveri.* Four chromosomes are evident in the early division phases of division I of meiosis (FIGS. 67, 68). At interphase between division I and II, the nucleolus becomes evident (FIGS. 69–71). Division II is reductional with four chromosomes appearing in early division phases (FIGS. 71, 72) and two chromosomes moving to each pole at anaphase (FIGS. 73, 74). This reduction division produces the four haploid nuclei (FIG. 75). A third equational division of the four haploid nuclei results in the eight ascospore nuclei (FIGS. 76–79).

Spores are delimited in a similar manner as in *T. carveri;* large portions of cytoplasm containing one or more nuclei are delimited by the fusion of vacuoles (FIGS. 69, 71, 78, 79). These eventually divide into uninucleate portions which develop cell walls and become the ascospores (FIGS. 80, 81).

Budding of the ascospores within the ascus is occasionally seen in this species. However, it occurs in much the same manner as in *T. carveri* where only a few blastospores are produced. The ascus does not become packed with the small bud cells.

Taphrina deformans (Berk.) Tulasne. The mycelium of this organism is both intercellular and subcuticular. Infection and subsequent development of the mycelium are produced by blastospores or possibly ascospores. Penetration occurs by means of a germ tube as described by Fitzpatrick (**2**) and Mix (**9**). The nucleus of the germinating spore undergoes a division, and the two daughter nuclei become a conjugate pair. The binucleate condition or dicaryophase is maintained by conjugate division throughout the vegetative mycelium.



FIGS. 54–59.

The penetration tube apparently enters the internal tissues and develops to form intercellular (FIG. 82) rather than subcuticular mycelium. The subcuticular mycelium is produced by hyphae from the intercellular mycelium growing out between the epidermal cell walls (FIG. 83). These hyphae form an anastomosing, richly branched system of mycelium. The hyphae of this stage are rather fine and composed of short cells (FIG. 109). As this mycelium matures, the cells of the hyphae become separated (FIG. 110) and enlarge into the typical irregularly shaped ascogenous cells (FIGS. 84–93).

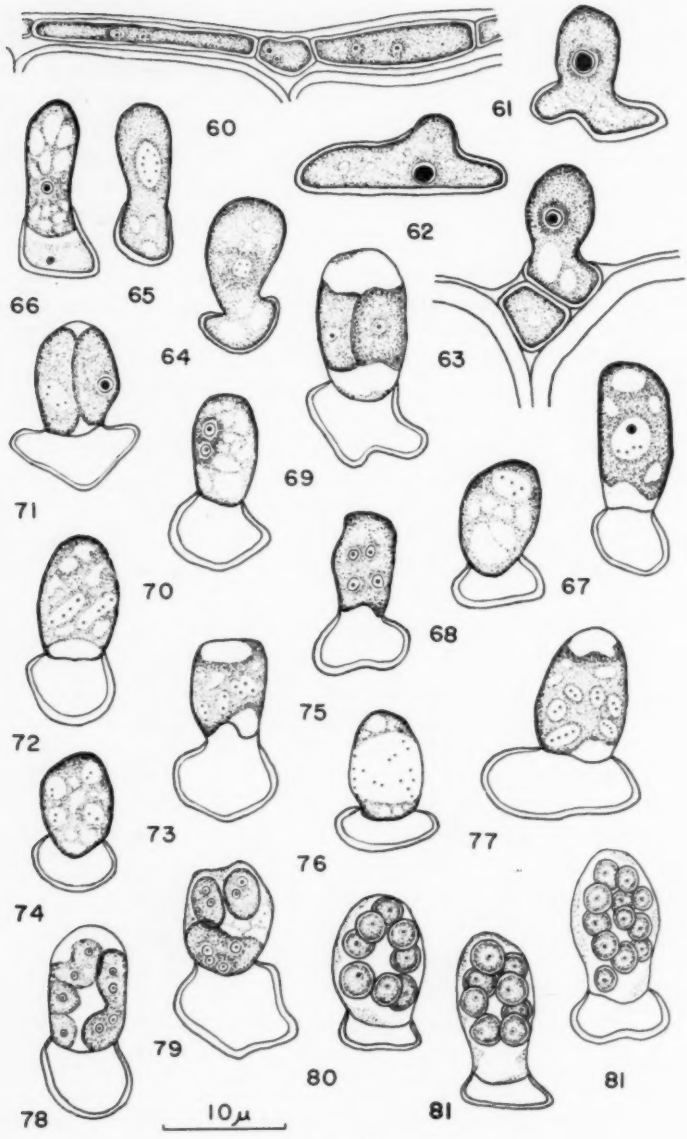
In the two species described earlier which have stalk cells, *T. carveri* and *T. ulmi,* the ascogenous cell wall ruptures and allows the inner membrane to emerge to form the ascus. This species illustrates a different type of ascus development. Instead of a papilla emerging to form the ascus, the entire ascogenous cell elongates, stretching the wall as it enlarges to form the ascus (FIGS. 89, 94, 95).

The first division of the fusion nucleus is equational, as described by Martin (**8**) with one of the resulting nuclei passing into the developing ascus while the other remains in the stalk cell (FIG. 95). The stalk cell is cut off in the same manner as described by Martin. The nucleus and cytoplasm of the stalk cell disintegrate (FIGS. 96, 97) as described in the species above.

Martin (**8**) reported a chromosome number of eight in the diplophase for this species. She illustrated an elongating ascogenous cell containing a fusion nucleus at anaphase with eight chromatids passing to each pole. The author, however, has never been able to see a nuclear division at this stage with sixteen chromatids. Four and eight are the only numbers that could be seen (FIGS. 90–94). This indicates a diploid chromosome number of four rather than eight as she proposed.

Meiosis, which occurs in the same manner as described for the species above (FIGS. 96–101), is followed soon after by an equational division that gives rise to the normal eight ascospore nuclei (FIGS. 102–104). Ascospore formation occurs as described by Martin (**8**) by the fusion of vacuolar membranes (FIGS. 105–108).

FIGS. 54–59. *Taphrina ulmi* (Fkl.) Johanson. × 930. FIG. 54. Intact ascogenous hyphae with some cells sterile and others bearing asci. FIG. 55. Young subcuticular mycelium in leaf cross section. FIG. 56. Cross section of young subcuticular mycelium. FIG. 57. Young, branching subcuticular mycelium on a portion of stripped host epidermis. FIG. 58. Hyphal tip of advancing mycelium at extreme edge of lesion. FIG. 59. Mature ascogenous mycelium on a portion of stripped host epidermis; some cells bearing asci.



FIGS. 60–81.

Taphrina populi-salicis Mix. The vegetative mycelium of this species is subcuticular in habit (FIGS. 112, 119, 120). It is not so extensive and freely branched as in some of the other species, such as *T. deformans;* however, branching does occur. Most of the cells composing the vegetative mycelium soon become enlarged to form the irregularly shaped ascogenous cells (FIGS. 121, 122).

The ascogenous cells appear to be formed in much the same manner as chlamydospores. When the cells enlarge, the cytoplasm may frequently be seen to contract into the swollen portion of the cell and form a secondary inner wall. When this occurs, either one or both of the empty ends of the original cell are cut off (FIG. 121). As these ascogenous cells mature, the connecting cell walls disintegrate leaving them free. During this time they begin to produce rhizophores that extend deep into the host tissue. These do not become septate, although they sometimes extend inward through three or four layers of host cells (FIG. 113).

The ascogenous cells are at first binucleate, as in the mycelium. However, fusion of the dicaryon soon occurs producing a large diploid nucleus (FIG. 112). During the elongation of the ascus, a septum is formed cutting off a stalk cell in the same manner as in the species discussed above (FIG. 114). A mitotic division of the fusion nucleus preceding the formation of this septum also occurs in this species. One of the nuclei migrates into the stalk cell producing a vegetative cell of the diploid generation (FIG. 113). On maturation of the ascus, a typically

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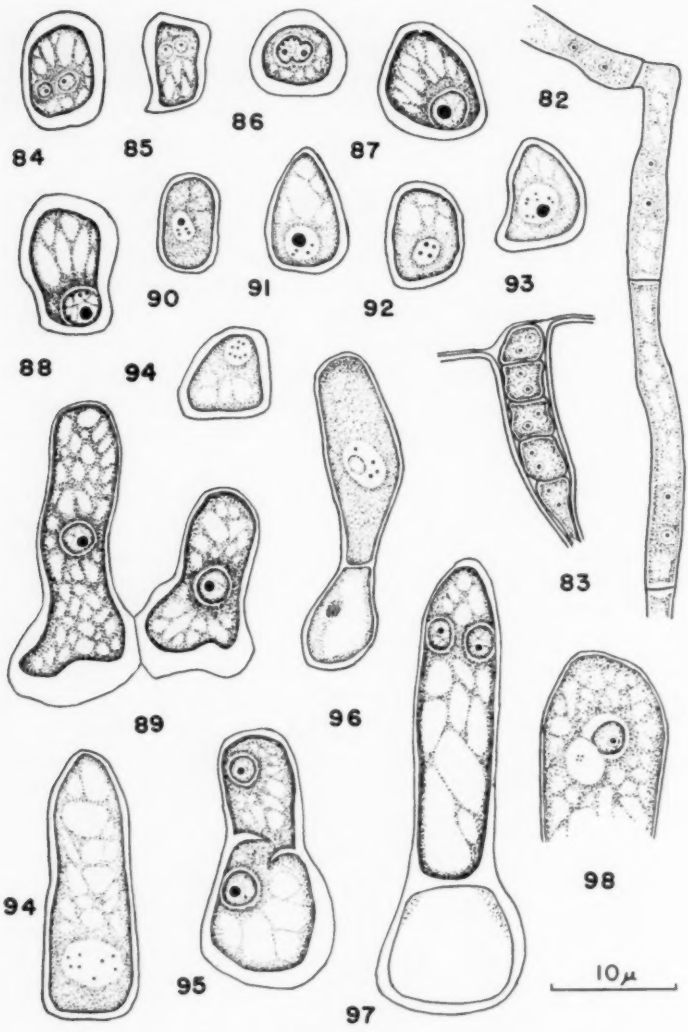
FIGS. 60–81. *Taphrina ulmi* (Fkl.) Johanson. × 1860. FIG. 60. Vegetative mycelium composed of binucleate cells. FIGS. 61–63. Papilla emerging from ascogenous cell containing large fusion nucleus. FIG. 64. Early division phase of fusion nucleus undergoing mitotic division; 4 chromosomes evident. FIG. 65. Later phase of same division showing 8 chromatids. FIG. 66. Young ascus with stalk cell containing diploid nucleus undergoing degeneration. FIGS. 67, 68. First meiotic division; 4 chromosomes evident in early division phase. FIG. 69. Two nucleate ascus; cytoplasm delimited into two large portions. FIG. 70. Two nucleate ascus. FIG. 71. Two nucleate ascus; one nucleus in interphase while the other may be interpreted as in FIG. 72. FIG. 72. Interpreted either as late phase of first meiotic division or early phase of second meiotic division. FIGS. 73, 74. Interpreted either as late phase of second meiotic division or early phase of third mitotic division; reduction to haploid number of chromosomes is complete with each of four nuclei containing 2 chromosomes. FIG. 75. Four nucleate ascus. FIGS. 76, 77. Equational division of 4 haploid nuclei, each nucleus receiving the haploid number of 2 chromosomes. FIGS. 78, 79. Eight nucleate ascus; cytoplasm of ascus being delimited into smaller uninucleate or multinucleate portions. FIGS. 80, 81. Mature asci, with 8 ascospores or with slight budding of ascospores.



FIGS. 82–98.

FIGS. 82–98. *Taphrina deformans* (Berk.) Tulasne. × 1860. FIG. 82. Intercellular, vegetative mycelium with binucleate cells. FIG. 83. Intercellular mycelium growing between host epidermal cells to become subcuticular. FIGS. 84, 85. Binucleate ascogenous cell. FIG. 86. Fusion of dicaryotic nuclei in ascogenous cell. FIGS. 87–89. Ascogenous cell containing fusion nucleus. FIGS. 90–92. Early division

empty stalk cell is left by the disintegration of its nucleus and cytoplasm (FIGS. 116–118).

The ascus in this species is formed by the stretching and elongation of the ascogenous cell wall. The wall was never observed to rupture to allow emergence of the inner membrane to form the ascus wall.

Although good material was available, consistent chromosome counts could not be obtained. Consequently, the chromosome number of this species is still uncertain.

It is assumed that meiosis occurs in the first two divisions of the fusion nucleus, as it does in the other species studied. Asci with 2, 4, and 8 nuclei were seen (FIGS. 115–117). The first indication of spore formation, however, was not observed until a rather large number of nuclei was produced. This indicates for the first time that nuclear divisions other than those normally required to produce the 8 ascospore nuclei occur. These nuclei are extremely small compared with those resulting from the first two or three divisions (FIG. 118).

Spore formation is apparently a progressive process. The cytoplasm of the young ascus is extremely vacuolate and forms a layer next to the wall. The first spores are usually formed in the tip with subsequent formation progressing toward the base of the ascus (FIG. 118). Occasionally, however, spore formation starts near the middle of the ascus. The number of nuclei present in the ascus, at the time when spore formation begins, appears to be far fewer than the number needed for the many spores of the mature ascus. Division of some nuclei apparently takes place coincident with the formation of spores by other nuclei. Eventually all of the nuclei enter into spore formation and the ascus becomes filled with spores.

DISCUSSION

Development of the mycelium and ascogenous layer. The development of the mycelium by different species of *Taphrina* has reached three distinct levels with regard to its location within the host tissue. These are the intercellular, subcuticular, and "cell wall" habits of development.

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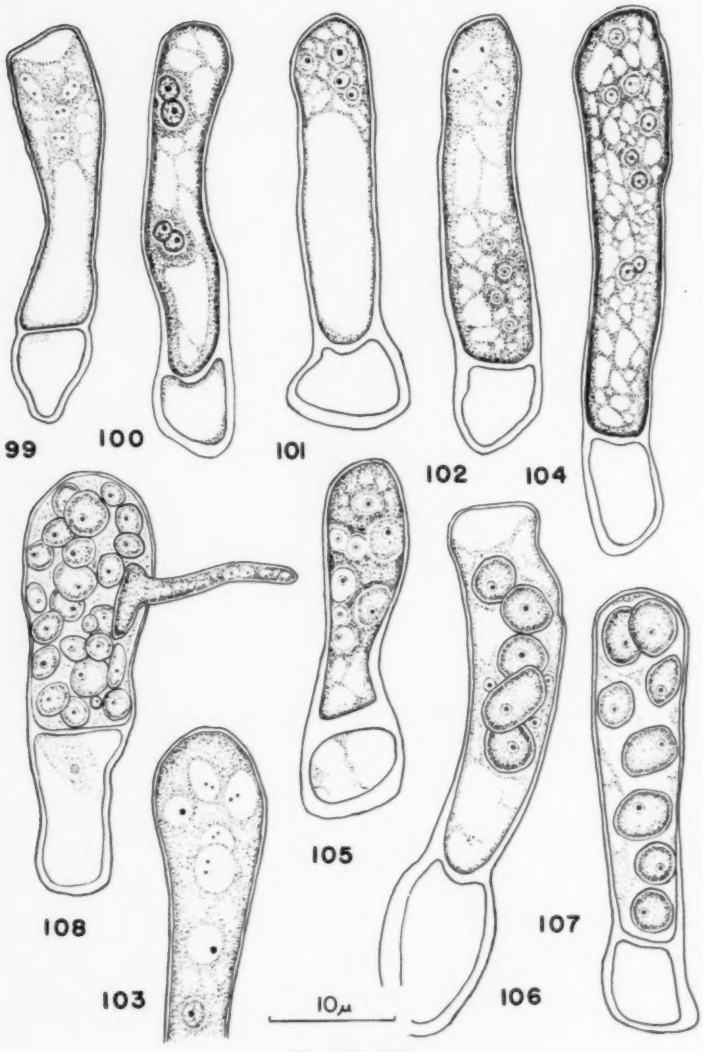
phase of equational division of fusion nucleus; 4 chromosomes evident. FIGS. 93, 94. Late division phase of equational division of fusion nucleus; 8 chromatids evident. FIG. 95. Formation of stalk cell by constriction of plasma membrane; stalk cell and ascus each with one diploid nucleus. FIG. 96. Early phase of first meiotic division of fusion nucleus, 4 chromosomes evident; nucleus and cytoplasm of stalk cell degenerating. FIG. 97. Binucleate ascus. FIG. 98. One nucleus in interphase, while the other undergoes division.



FIGS. 99–108.

FIGS. 99–108. *Taphrina deformans* (Berk.) Tulasne. × 1860. FIG. 99. Interpreted as late phase of second meiotic division or early phase of third mitotic division. FIGS. 100, 101. Four nucleate ascus. FIG. 102. Ascus with four ascospore nuclei, and 2 nuclei undergoing equational division to form the other 4

Among the species with an intercellular mycelial habit, those, such as *T. potentillae,* do not produce a subcuticular layer of ascogenous cells, but instead bear their asci at the tips of branches of the intercellular mycelium that grow outward between the epidermal cells. In other species, such as *T. deformans,* branches of the intercellular mycelium become subcuticular and branch out over the surface of the host epidermal cells (FIG. 109). This subcuticular mycelium then undergoes precocious branching, becomes highly septate and finally fragments to produce a layer of individual ascogenous cells, the hymenium.

In a great many species, the mycelium is entirely subcuticular, not becoming intercellular. The two species, *T. ulmi* and *T. celti* that occur on members of the Urticaceae, have a subcuticular mycelium that remains intact throughout and, after the development of the asci, not breaking into individual ascogenous cells. It is not extensively branched and only certain cells are functional in producing asci; many remain sterile. A somewhat similar type of mycelial and hymenial development, in which the mycelium generally remains intact for some time while the cells enlarge and in which some of the cells remain sterile, is exhibited by *T. populi-salicis.*

In a large number of species, such as *T. epiphylla* and *T. tosquinetii,* an ascogenous layer is produced in the same manner as *T. deformans,* but from mycelium that is subcuticular from its beginning, never having been intercellular. In the type of development described above for *T. virginica* there is a further reduction in mycelial formation. In this species the only mycelium that develops is that referred to as "distributive hyphae." The hymenium develops from a colony of budding yeast-like cells rather than fragmentation of hyphae.

In the third major type of mycelial and hymenial development, which Mix (**10**) termed the "wall-habit" form, the hyphae enter the wall of the epidermal cells and form a locule between the layers of the cell wall. In this position, the mycelium fragments to form a group of ascogenous cells.

Nuclear behavior. Certain aspects of the cytology of this genus have for some time been controversial. This has been mainly centered around the division of the fusion nucleus in the young ascus and the subsequent course of development of the ascus in species with stalk cells.

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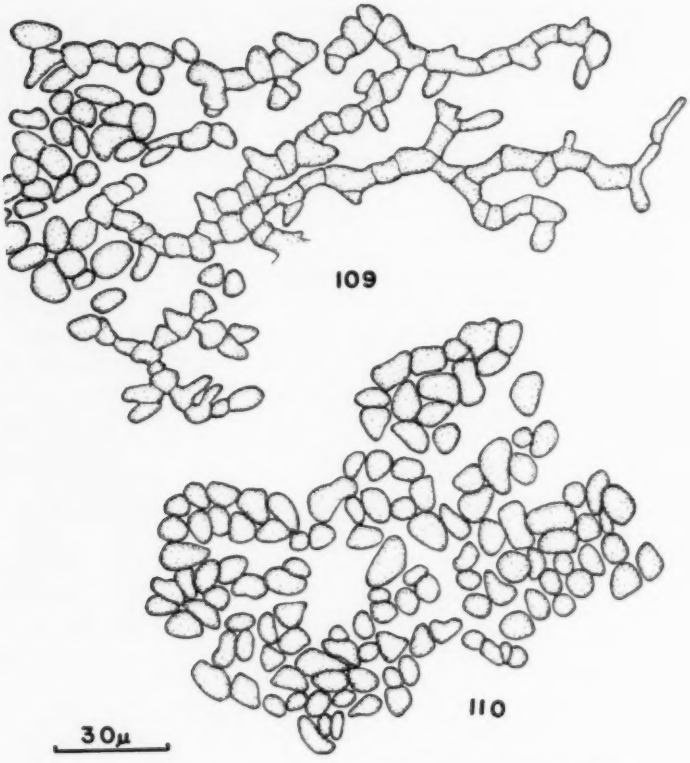
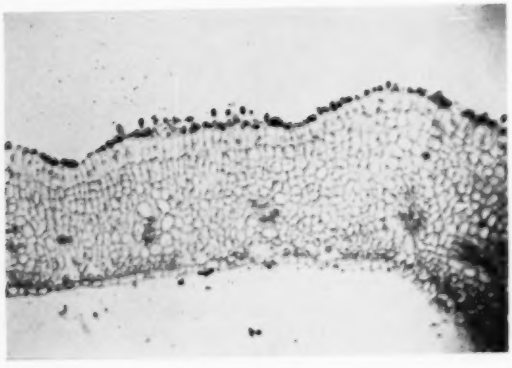
ascospore nuclei. FIG. 103. Ascus with nuclei in various stages of division. FIG. 104. Ascus with 8 haploid nuclei. FIG. 105. Formation of 8 ascospores. FIG. 106. Only 5 ascospores formed; 3 nuclei not entering into spore formation. FIG. 107. Mature ascus. FIG. 108. Blastospore formation; germination of a spore to form a germ tube with conjugate pair of nuclei.





FIGS. 109–111.

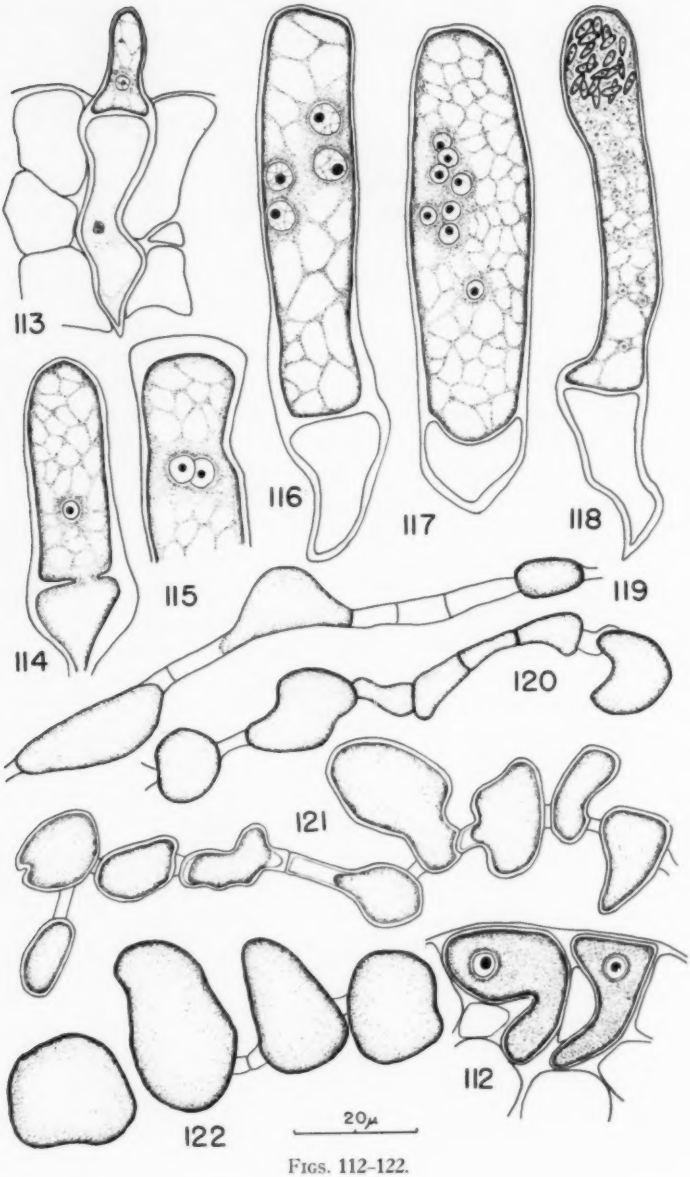
Through the work of Ikeno (3), Fitzpatrick (2), Mix (9), and others, it is generally accepted that the dicaryotic condition of the nuclei, which has been found to be present in all species studied, arises through a mitotic division of the ascospore or blastospore nucleus at the time of germination. This dicaryotic condition is maintained by subsequent conjugate nuclear divisions. The binucleate condition remains throughout the development of the organism and is not concluded until nuclear fusion occurs in the ascogenous cells. The fusion nucleus is large and contains a prominent nucleolus.

As the ascogenous cells mature and enter the formation of an ascus, the fusion nucleus undergoes division. Many workers, including Juel (7), Eftimiu (1), and Mix (9), believed that this was the first division of meiosis. Under this assumption they also believed the stalk cell, when present, was cut off without contents. However, Ikeno (3) and Martin (8) found that in the species they studied which had stalk cells, the first division was mitotic. This also has been found to be true in the species with stalk cells investigated here, but not in those without stalk cells. In species with stalk cells, one of the nuclei resulting from this mitotic division of the fusion nucleus migrates into the papilla which is to become the ascus, while the other remains in the basal portion of the ascogenous cell. After this division, a cross wall is formed that cuts off the stalk cell containing one of the nuclei and some of the cytoplasm. Coincident with the development of the ascus, the nucleus and protoplasm of the stalk cell disintegrate leaving the stalk cell empty.

This equational division is an important feature, since it produces a vegetative diploid cell, the occurrence of which is quite rare in the fungi. To my knowledge, the only other Ascomycetes where such a cell or "generation" may be found is in certain members of the Endomycetales.

In the species *T. virginica,* which lacks a stalk cell, this mitotic division preceding meiosis does not occur. Instead, the fusion nucleus undergoes immediate meiosis. This is also thought to occur in *T. communis, T. flavorubra,* and *T. purpurascens* which have been studied but are not reported here. Whether this will hold true for all species with-

FIGS. 109–111. *Taphrina deformans* (Berk.) Tulasne. FIGS. 109, 110, × 425. FIG. 111, × 41. FIG. 109. Ramifying subcuticular mycelium from portion of stripped host epidermis at margin of lesion; part of mycelium segmenting to form ascogenous cells. FIG. 110. Ascogenous layer on portion of stripped host epidermis. FIG. 111. Cross section of leaf showing fungus in various stages of development.



FIGS. 112-122.

out stalk cells is hoped to be determined in the future. Another question that arises at this point concerns several species, such as *T. johansonii* and *T. populina,* that have asci which may or may not have stalk cells. It would be extremely desirable to know whether these two different nuclear cycles occurred in the same species, depending on whether a stalk cell was produced.

In the species studied without stalk cells, the fusion nucleus undergoes the same processes toward spore formation as does the ascus nucleus derived from the first mitotic division in species with stalk cells. Meiosis is followed by an equational division to produce 8 ascospore nuclei. The only known exception to this is in *T. populi-salicis* where continuous mitotic divisions of the haploid nuclei produce a large number of ascospore nuclei. It is not known at this time whether this process also occurs in its closely related species *T. populina, T. johansonii,* and *T. rhizophora,* as well as in others such as *T. kusanoi, T. bacteriosperma,* and *T. carnea.* However, Mix (**11**) made the statement for each of these and others that "ascospores were not seen" or "ascospores are rarely seen, budding at once to fill the ascus with numerous blastospores," indicating the possibility of this type of spore formation being present. Mix did not study these organisms cytologically and would therefore have been unaware if numerous spore nuclei were produced.

Eftimiu (**1**) reported the haploid number of chromosomes to be 2 while Martin (**8**) reported 4 in both *T. deformans* and *T. coryli.* In the present study, the chromosome number was found to be 2 in the haploid and 4 in the diploid condition. This was true of all species studied.

Ascospore formation. Spore formation in these organisms occurs through the fusion of vacuolar membranes. Portions of cytoplasm containing a single nucleus become surrounded by small vacuoles and are delimited when the inner membranes unite. After formation of a wall, this portion of cytoplasm becomes an ascospore which is always uninucleate.

FIGS. 112–122. *Taphrina populi-salicis* Mix. × 930. FIG. 112. Ascogenous cells with large fusion nucleus. FIG. 113. Young uninucleate ascus, with stalk cell also containing a single nucleus in a stage of degeneration. FIG. 114. Formation of stalk cell by constriction of plasma membrane. FIG. 115. Binucleate ascus. FIG. 116. Four nucleate ascus. FIG. 117. Eight nucleate ascus. FIG. 118. Ascus with numerous nuclei, some of which have undergone spore formation. FIGS. 119, 120. Young, vegetative, subcuticular mycelium undergoing formation of ascogenous cells. FIG. 121. Formation of ascogenous cells. FIG. 122. Mature ascogenous cells as they appear on a portion of stripped host epidermis.

Very often, preceding spore formation, the cytoplasm divides into rather large portions, occasionally containing several nuclei. As nuclear divisions proceed, they are further divided until uninucleate portions result and become ascospores.

After spore formation there is a small amount of epiplasm left in the ascus. Although Martin (**8**) also believed that the spores were delimited by fusion of vacuolar membranes, she mentioned that the presence of epiplasm might indicate that the spores were produced by beak formation.

SUMMARY

The developmental morphology and nuclear behavior of 5 species, which are considered to represent some of the major morphological types within the genus *Taphrina*, have been studied. The development of the mycelium by members of the genus has attained three distinct levels with regard to its location within the host tissue. These are the intercellular, subcuticular, and cell wall habits of development. Only members with the first two types have been studied at this time. These and the variations that exist among them may be summarized as follows:

1. Intercellular mycelium that does not form a subcuticular layer, but instead bears asci at the tips of hyphal branches which grow outward between the epidermal cells, such as found in *T. caerulescens* and *T. carnea;*

2. Mycelium which is at first intercellular, but becomes subcuticular and fragments to form a hymenium of separate ascogenous cells, such as found in *T. deformans;*

3. Mycelium that is only subcuticular in habit and which remains intact throughout the development and maturation of the asci; (Not all of the cells enter into ascus formation, some remain sterile. Only 2 species, *T. ulmi* and *T. celtii,* exhibit this type of mycelial development.)

4. Subcuticular mycelium which is sparingly branched and which remains intact during the early development of the ascogenous cells; (As in the above type, not all cells enter ascus formation. This type of mycelial formation may be found in *T. populi-salicis.*)

5. Subcuticular mycelium that is richly branched and which fragments into a compact hymenium of ascogenous cells, such as found in *T. carveri* and *T. betulae;*

6. Hymenia which are formed by subcuticular colonies of budding yeastlike cells which enlarge to form a compact layer of ascogenous cells; (Mycelium is only sparsely produced, functioning mainly in distributing the fungus over the leaf surface.)

7. Mycelium that grows within the walls of the epidermal cells and locules in which the ascogenous cells and asci develop.

Three distinct patterns of nuclear behavior are now evident in the genus. They are as follows:

1. Species with stalk cells in which the fusion nucleus undergoes an equational division preceding meiosis. One of the resulting nuclei migrates into the stalk cell and disintegrates while the other undergoes meiotic division and finally produces 8 ascospores. This type of nuclear behavior occurs in all species with stalk cells that have been studied with the exception of *T. populi-salicis*.

2. The species *Taphrina virginica*, which lacks a stalk cell, possesses a nuclear cycle that is the same as that described above except that the fusion nucleus undergoes immediate meiosis and spore formation without a preceding equational division. The extent to which this type of nuclear cycle occurs among those organisms is not known, although it is suspected that it does occur in at least some other species which lack stalk cells.

3. The species *Taphrina populi-salicis* has a nuclear behavior that is the same as the first type mentioned above, except that nuclear division does not stop after eight haploid nuclei are formed but continues indefinitely to produce a large number of ascospore nuclei. This is the only species in which this type of nuclear cycle is known to occur; however, it seems quite possible that the same phenomenon may occur in other species.

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RUST OF THE SMOKE-TREE[1]

G. E. THOMPSON

(WITH 14 FIGURES)

In 1956, I (**4**) reported the discovery of an autoecious rust on the smoke-tree, *Cotinus coggygria* Scop. (*Rhus cotinus* L.) in Athens, Georgia, described the symptoms of the disease, and recorded observations on the aecia, uredinia, and telia. The rust was identified provisionally as *Pileolaria cotini-coggyriae* described by Tai and Cheo (**3**) in 1937 from collections made in China.

The rust was found on a tree planted on the campus of the University of Georgia about 45 years ago. It has not been found in other localities in North America.

This paper amends the original diagnosis by describing the spermagonia, aecia, and basidia, by redescribing the uredinia, and by reporting observations made on the life cycle of the fungus. The spelling of the species name is also corrected from *"cotini-coggyriae"* to *"cotini-coggygriae."*

The type specimen, obtained from the Arthur Herbarium, Purdue University, Lafayette, Ind., yielded no spores corresponding to the description of the urediniospores. But both urediniospores and teliospores are similar to those of the Athens' collections, so that the two rusts are apparently identical. Spermagonia and aecia are not present on the type.

The species is redescribed as follows:

Pileolaria cotini-coggygriae Tai & Cheo (Bull. Chinese Bot. Soc. **3**: 59. 1937) emend. Thompson

Spermogonia amphigenous, petiolicolous, caulicolous, or floricolous, in hypertrophied areas, subcuticular, conical 50–100 × 33–40 μ; spermatia elliptical, hyaline, 3–3.5 × 4.5–6 μ; aecia hypophyllous, petiolicolous, caulicolous or floricolous, in hypertrophied areas, subepidermal, erumpent, confluent, covering extensive areas, pulverulent, cinnamon-brown; aeciospores mostly ovoid or pyriform, occasionally globose, minutely echinulate, pale brown, 20–26 × 26–36 μ, pores 4–5, chiefly

[1] Journal Paper No. 132 of the College Experiment Station of the University of Georgia College of Agriculture Experiment Stations.

equatorial, wall about 3.5 μ thick, borne singly on hyaline pedicels, 6–6.5 × 30–50 μ; uredinia chiefly hypophyllous, subepidermal, erumpent, in minute dark spots .25–.5 mm diam; urediniospores pulverulent, brown, globose, 20–26.5 μ, pale brown, minutely echinulate, pores 4–5, scattered, wall about 3.5 μ thick, borne singly on hyaline pedicels, 6–7 × 16–20 μ; telia chiefly epiphyllous, subepidermal, erumpent, chocolate-brown; teliospores globose or depressed globose, 30–36 × 26–36 μ, wall smooth or slightly roughened, dark brown, wall about 6.5 μ thick, single apical pore, pedicel hyaline, 6.5–7 × 64–140 μ, lower half spiculate; basidiospores hyaline, ovoid or subglobose, apiculate, 6.5–7 × 7–9.5 μ.

Specimens have been deposited in the Arthur Herbarium, Purdue University, Lafayette, Indiana and in the Mycological Herbarium, University of Georgia, Athens, Georgia.

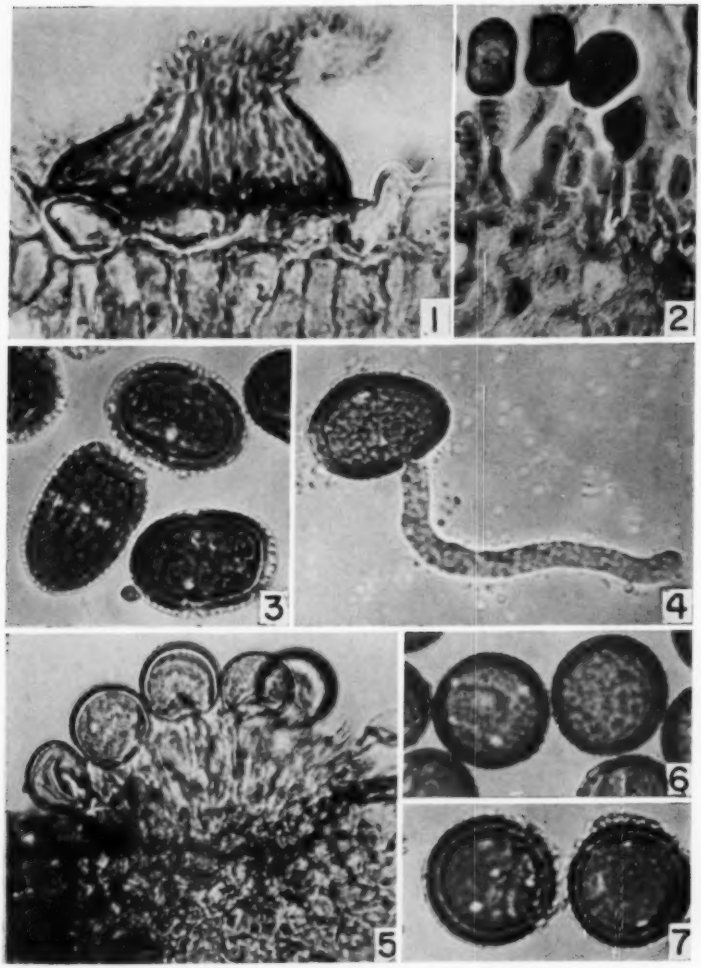
OBSERVATIONS ON THE LIFE CYCLE

Leaves bearing telia were collected in the fall of 1957 and 1958 and placed under a wire screen frame for over-wintering. Between March 15 and April 15 of the following year the leaves were examined after rains for germinating teliospores. During the same period leaves were collected from the same source, moistened, and placed in moist chambers. Also, teliospores were transferred from leaves to water drops on slides.

Teliospores germinated within 8–12 hours under each of the above conditions. However, normal germination of the teliospores was observed most frequently on the leaves which were collected out-of-doors shortly after rains. A single, hyaline, 3-septate basidium, 6–6.5 × 15–95 μ, developed from the apical pore of the teliospore (FIG. 12). The hyaline, ovoid to subglobose, apiculate basidiospores, 6.5–7 × 7–9.5 μ (FIG. 12) were produced at the tips of the pointed sterigmata (FIG. 13). Basidia formed from teliospores on leaves in moist chambers and in water drops were mostly atypical and developed vacuolated tubelike structures, 240 μ or more long. The sterigmata, instead of bearing basidiospores, continued to elongate into hyphae about 3.5 μ diam. In other cases, two or more germ tubes arose from the cells of the basidia. These plasmolyzed and degenerated.

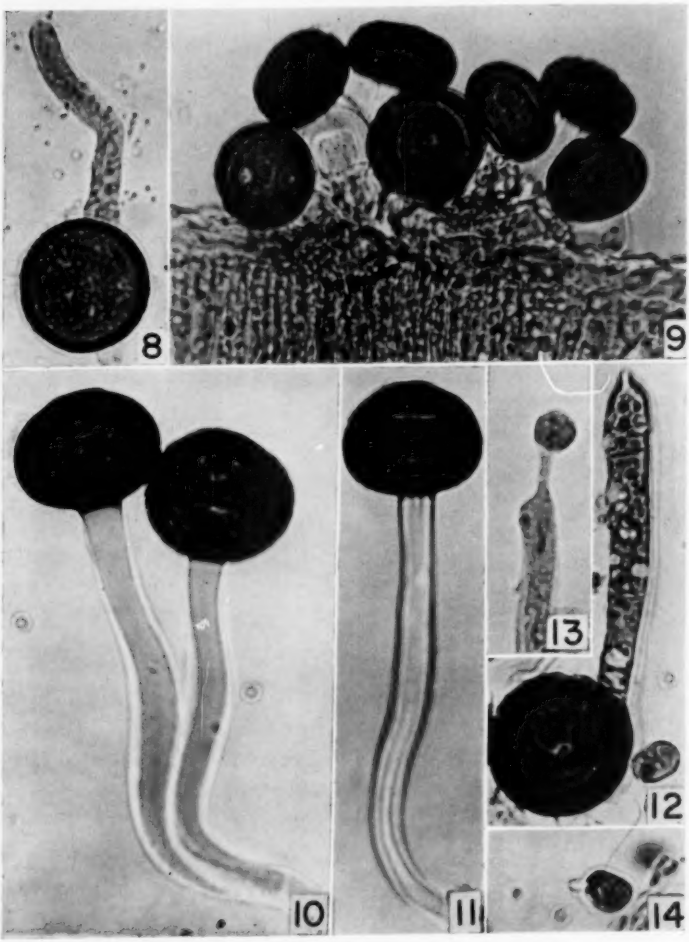
Although not established experimentally, the basidiospores which mature during the last of March and early April probably initiate the primary infections. These occurred on twigs, leaf blades and petioles, rachises, sepals, and young ovaries. The lesions were reddish in color and the affected organs became overdeveloped and distorted.

Spermagonia (FIG. 1) developed shortly after infections became visible. The earliest ones were observed on April 5. They occurred on



FIGS. 1–7. *Pilcolaria cotini-coggygriae*. FIG. 1. A spermagonium with extruded spermatia. × 725. FIG. 2. A portion of an aecium with mature and immature binucleate aeciospores and stalk cells. × 335. FIG. 3. Aeciospores with equatorial germ pores. × 670. FIG. 4. Germinated aeciospore. × 670. FIG. 5. Uredinium. × 445. FIG. 6. Urediniospores with scattered germ pores. × 670. FIG. 7. Urediniospores from Chinese specimens. × 670.

both sides of the leaf and encircled the lesions on shoots. The spermatiophores, 3.5 × 15–25 μ, were widest at the base and tapered to the tip. In young spermagonia they converged toward a central pore and, as the spermagonia spread laterally, became more erect and the opening en-



FIGS. 8–14. *Pileolaria cotini-coggygriae*. FIG. 8. Germinated urediniospore. × 670. FIG. 9. Telium. × 450. FIG. 10. Teliospores. × 670. FIG. 11. Teliospore from Chinese specimens. × 670. FIG. 12. Germinated teliospore with 3-septate basidium and mature basidiospore (lower right). × 670. FIG. 13. Immature basidiospore at tip of terminal sterigma. × 670. FIG. 14. Germinated basidiospore. × 670.

larged, giving the aspect of an acervulus. The spermatia appeared to be abstricted from the tips of the spermatiophores. Prior to the extrusion of the spermatia, a colorless droplet formed at the pore of the spermagonium, but it became milky when filled with spermatia.

Aecia with aeciospores (FIG. 2) developed 2–3 weeks after the spermagonia and were always associated with hypertrophied tissue. The earliest collections were made on April 23. They were subepidermal and numerous, but as they spread laterally, they coalesced and finally covered the underside of leaf infections or encircled infected shoots and floral parts. Peridia and paraphyses were lacking. Aeciospores dusted into water drops and kept in moist chambers formed a single hyaline germ tube about 3.5 μ diam (FIG. 4).

Uredinia (FIG. 5) developed about 3–4 weeks after the aecia. The earliest collections were on May 21. The uredinia were chiefly on the lower side of the leaves in minute reddish spots which, when numerous, coalesced to form extensive discolored areas. A few uredinia occurred on the rachises of the inflorescences. Unlike the aecia, the uredinia did not spread laterally and fuse but remained separate.

The urediniospores were described by Tai and Cheo as ellipsoid, 30–48 × 22–26 μ, verrucose in straight rows and with 4–5 equatorial pores. Such spores were not found on the type but urediniospores similar to those on the Athens' collections were present (FIGS. 6, 7). Most species of *Pileolaria* (**1**, **2**) have verrucose markings arranged in rows. In this species the surface is minutely echinulate and the pores are scattered instead of being equatorial.

Urediniospores germinated in water by a single germ tube which usually arose from a germ pore located near the hilum (FIG. 8). Urediniospores were germinated at three temperatures. At the end of six hours, the average length of the germ tubes at 20° C was 64 μ; at 25° C, 127.5 μ; and at 30° C, 73 μ.

The first telia (FIG. 9) associated with uredinia were observed on June 21. They increased in abundance as the season advanced. A comparison of teliospores from the type with those from Athens showed them to be similar. They were described originally as being verrucose, but the majority appeared to be almost smooth or only very slightly roughened (FIGS. 10, 11). Most of the teliospores remained *in situ* on the leaves during the winter; some became detached and scattered over the leaf surface, but a few were observed lying on the bark of twigs.

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A NEW GENUS OF THE MELIOLACEAE ON THE ROOTS OF SOLANUM SPECIES [1, 4]

C. C. GORDON [2] AND CHARLES GARDNER SHAW [3]

(WITH 2 FIGURES)

INTRODUCTION

A superficial blackening of the fibrous root system of potatoes, *Solanum tuberosum* L., has been observed annually since 1944 (**1**, **3**) in a series of crop rotation plots at the Irrigation Experiment Station, Prosser, Washington. Although conspicuous on the fibrous roots, the mycelium has never been observed on the tubers or on above-ground parts of potato plants. It has been reported previously (**1**, **3**) that this discoloration is due to a dense reticulum of dark brown hyphae of a fungus that is apparently an undescribed member of the Meliolaceae. Since most of the known species of this family occur on the leaves of tropical plants, the occurrence of a black mildew (**5**) on roots in a temperate region prompted detailed study of its morphology and taxonomic relationships.

MORPHOLOGY

The mycelium (FIG. 1, A) bears numerous lateral and terminal, 2-3-celled, capitate hyphopodia. From the terminal cell of each hyphopodium a penetration peg (FIG. 1, I) is produced which penetrates the cell wall of a root epidermal cell. Frequently the penetration peg itself apparently functions as a haustorium, and might be termed a stylet-type haustorium (FIG. 1, J). Less often the tip of the penetration peg enlarges to form a small, bulbous haustorium (FIG. 1, K).

[1] Scientific Paper No. 1984, Washington Agricultural Experiment Station, Pullman, Wash., Proj. 9061. The assistance of C. G. Hansford is gratefully acknowledged.

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[4] Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, Washington State University.

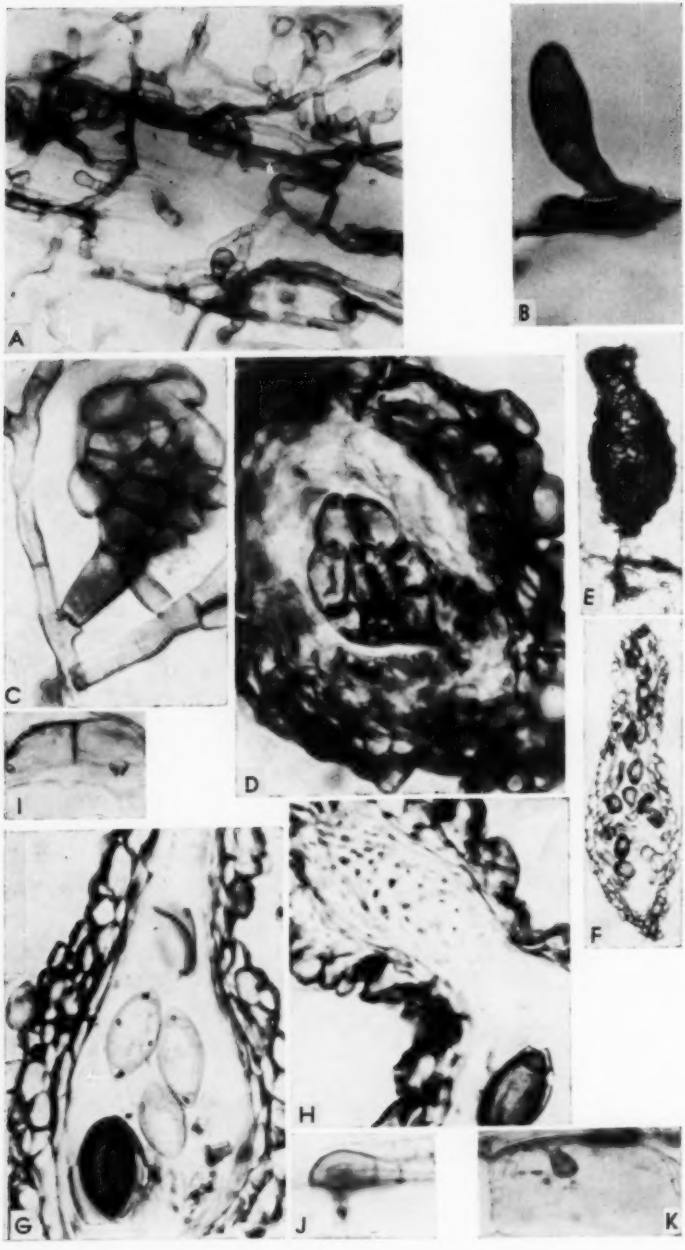

FIG. 1.

Collapse of numerous root epidermal cells, followed by death, occurs in the area of mycelial development. Subsequently hyphae encroach into the lumina of the dead epidermal cells and produce hyphopodia which send penetration pegs into the outermost layer of the cortex. Cells of this layer of the cortex in turn may collapse and be invaded by hyphae. Penetration pegs and haustoria have been observed in the second layer of the cortical cells, but not in deeper layers.

Superficial perithecia (FIG. 1, E) are produced profusely on the mycelium. The youngest stage found consists of an upright 3-celled mycelial branch (FIG. 1, B). The terminal cell divides in three planes producing a spherical mass of black angular cells (FIG. 1, C). Initially the young perithecium is filled with hyaline parenchymatous tissue, which slowly disappears as the asci develop (FIG. 1, D). No paraphyses or pseudoparaphyses are found, although periphyses are present in the neck of the perithecium (FIG. 1, H). The wall of the mature perithecium is 2–5 cells in thickness (FIG. 1, F, G).

The asci are thin-walled and ephemeral, disintegrating before the ascospores are completely differentiated (FIG. 1, G). The 8-spored asci (FIG. 1, D) are globose to obovoid and are formed successively. Immature and mature ascospores are found in the same perithecium (FIG. 1, F, G).

The oval, immature ascospores are 4-nucleate (FIG. 1, G). A septation is formed near each apex of the spore cutting off two small apical cells, each containing one nucleus, and leaving two nuclei in the large central cell.

Although no sheath is visible around developing ascospores prior to their release from the asci, a wide hyaline sheath is obvious once the ascus has disintegrated (FIG. 2, A). This sheath is peculiar in that it does not completely enclose the ascospore, but contains two apical pores. As the ascospores mature, the sheath and the wall of the spore become

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FIG. 1. *Diporotheca rhizophila*. A. Black, septate, superficial mycelium bearing capitate hyphopodia, × 250. B. 3-celled perithecial initial, × 750. C. Multicellular perithecial primordium, × 600. D. Mature ascus containing eight immature ascospores, × 500. E. Surface view of mature perithecium. Note supporting "hyphal struts," × 90. F. Transverse section of mature perithecium containing ascospores, × 140. G. Transverse section of mature perithecium containing both mature and immature ascospores, × 500. Note 4-nucleate condition of the immature ascospore. H. Ostiolar region of the perithecium showing periphyses, × 500. I. Transverse section of a hyphopodium showing the penetration peg, × 1100. J. Transverse section of a hyphopodium showing a stylet-type haustorium, × 800. K. Bulbous haustorium within host epidermal cell, × 500.

intensely pigmented, finally being dark brown in color (FIG. 2, B). The ascospores are released passively through the ostiole (FIG. 1, F).

TAXONOMIC POSITION

Initial attempts to classify the fungus were unsuccessful because it was not recognized as an ascomycete. The basic clew was provided when, after repeated crushing of fruiting structures in fresh mounts, perithecia which contained eight and sixteen mature spores were encountered. The significance of the hyphopodia was then recognized and it became apparent that the fungus most logically should be associated with the Meliolaceae, to which the common name "black-mildews" has been given (**5**).

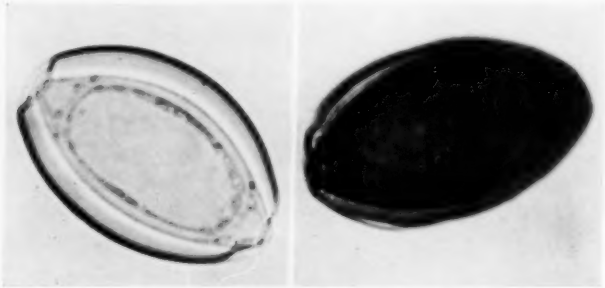

FIG. 2. *Diporotheca rhizophila*. A. Immature ascospore showing poroid sheath, × 1200. B. Mature, 2-septate ascospore, × 1200.

This fungus, however, differs markedly from any previously described species of the Meliolaceae (**2**, **4**, **5**). Hansford (**2**), in his description of the family, emphasizes the fact that all known members of the family are parasitic on the foliage and young twigs (occasionally on fruits and mature stems [5]) of higher plants. No member of the family has been described from the roots of plants. The incomplete sheath around the ascospore constitutes the second unique feature of this fungus. Other characters of significance are: (1) the 2-septate nature of the ascospore, (2) the disparity in size between the central cell of the ascospore and the two terminal cells, (3) lack of constriction in the outer wall of the ascospore at the septa, and (4) the well developed neck and periphysate, ostiolar region of the perithecium. The occurrence of this fungus in temperate regions, while not unique, is also worthy of men-

[5] *Fide* C. G. Hansford personal correspondence.

tion. The combination of characters necessitates the description of a new genus for this fungus. Since the persistent sheath with terminal pores is probably its most unusual feature, the generic name *Diporotheca* is proposed.

In spite of its unusual characters, the presence of capitate hyphopodia comparable to those found in many genera and species of the Meliolaceae justify the inclusion of *Diporotheca* in that family, even though the description of the family must be emended to include the rhizophilous habitat of this species.

DIPOROTHECA gen. nov.[6]

Mycelio superficiali; hyphis septatis, atro-brunneis, setis mycelialibus nullis; hyphopodiis capitatis; peritheciis atris, superficialibus, piriformibus, ostiolatis, glabris, aparaphysatis sed periphysatis; pariete peritheciali 2–5-stratoso, parenchymatico, membranaceo; ascis evanescentibus, octosporis; sporidiis brunneis, 2-septatis, in membrana pallidula brunnea poro apicibus ambopus penetrata inclusis; cellula media maiore.

Mycelium superficial, hyphae septate, dark brown, nonsetoid, bearing only capitate hyphopodia; haustoria originating from the terminal cells of the hyphopodia; perithecia black, superficial, supported by "hyphal struts," unappendaged, pyriform to flask-shaped, ostiolate, aparaphysate but with periphyses; perithecial wall parenchymatous, 2–5 cells thick, membranous; asci unitunicate, ephemeral, 8-spored; ascospores brown, 3-celled, the central cell much the larger, sheathed; sheath poroid at each apex.

Diporotheca rhizophila sp. nov.

Coloniis superficialibus in radicibus Solani spp.; mycelio reticulato ex hyphis brunneis, 3.4–4.2 μ crassis, septatis (cellulis 30–55 μ longis), irregulariter angulis rectis ramosis composito; setis mycelialibus nullis; hyphopodiis solum capitatis, 1–2-septatis, apice rotundatis, irregulariter dispositis, rectis vel curvatis, 13–22 $\times$ 6.5–12 μ; peritheciis superficialibus, numerosis, dispersis, atris, piriformibus, ostiolatis, aparaphysatis sed periphysatis, 240–400 $\times$ 110–175 μ; pariete peritheciali parenchymatico, membranaceo, 2–5-stratoso, extus atrobrunneo, intus hyalino, cellulis externis angulosis, 7–15 $\times$ 10–20 μ; rostris 117 longis $\times$ 65 μ crassis; ascis evanescentibus, globosis vel obovoideis, 25–40 $\times$ 50–75 μ, octosporis; ascosporidiis atrobrunneis, ovatis, 2-septatis, apice rotundatis, 30–35 $\times$ 16–20 μ, in membrana pallidula brunnea inclusis; cellulis inaequalibus; cellula media 20–25 μ longa, cellulis terminalibus 4–6 μ longis; membrana 0.8–1.7 μ crassa, poro apicibus penetrata.

Hab.: in radicibus Solani melongenae, S. tuberosi, et S. nigri.
Loc.: Benton, Yakima, et Kittitas Cos., WASHINGTON, U.S.A.

[6] The assistance of Donald P. Rogers in preparation of the Latin descriptions is gratefully acknowledged.

Typus est in rad. Solani melongenae; Coll. Prosser, Benton Co., Wash., Sept. 15, 1958, C. C. Gordon. (WSP #48900[7]).

Colonies superficial on and covering the fibrous roots of *Solanum* spp.; mycelium nonsetoid, producing a dense reticulum composed of septate dark brown hyphae, branching irregularly at acute angles, 3.4–4.2 μ wide, cells variable in length, mostly 30–55 μ. Hyphopodia capitate, 2–3-celled, arising irregularly, straight, curved or bent, 13–22 × 6.5–12 μ, the 3-celled hyphopodia being the longer; terminal cell the wider (widest). Mucronate hyphopodia lacking. Haustoria hyaline, peglike, or more rarely bulbous. Perithecia numerous, superficial, scattered, black, pyriform to flask-shaped, supported by "hyphal struts," ostiolate, 240–400 × 110–175 μ, neck averaging 117 μ in length and 65 μ in width, aparaphysate, periphyses lining the interior of the neck; perithecial wall 2–5 cells thick, membranous, not carbonaceous; surface cells irregularly angular in outline, 7–15 × 10–20 μ. Asci unitunicate, thin-walled, ephemeral, globose to obovoid, 25–40 × 50–75 μ, 8-spored. Ascospores dark brown, ovate, 3-celled, sheathed, 30–35 × 16–20 μ excluding the sheath; central cell 20–25 μ in length, terminal cells 4–6 μ in length, apically rounded; sheath light brown, 0.8–1.7 μ in thickness, encasing the spores except at the apices of the terminal cells. Parasitic on roots of *Solanum melongena* L., *S. tuberosum* L., and *S. nigrum* L.

TYPE COLLECTION: ON: *Solanum melongena* L.; Irrigation Experiment Station, Prosser, Benton Co., Washington, U.S.A.; Sept. 15, 1958; *C. C. Gordon* (WSP #48900).

PARATYPES: ON: *Solanum melongena* L.; Same locality; Oct. 2, 1958; *J. D. Menzies* (WSP #48898). Plant Pathology Greenhouses, Wash. State Univ., Pullman, Whitman Co., Wash. (Grown in soil from Irr. Exp. Sta., Prosser); May 23, 1960; *C. G. Shaw* (WSP #48903).

ON: *Solanum tuberosum* L.; Irr. Exp. Sta., Prosser, Benton Co., Wash. Summer, 1949; *J. D. Menzies* (WSP #21801). Same loc.; Summer, 1955; *J. D. Menzies* (WSP #48899).

ON: *Solanum nigrum* L.; Irr. Exp. Sta., Prosser, Benton Co., Wash.; Sept. 15, 1958; *C. C. Gordon* (WSP #48904). Plant Pathology Greenhouses, Wash. State Univ., Pullman, Whitman Co., Wash. (Grown in soil from Irr. Exp. Sta., Prosser); June 10, 1960; *C. C. Gordon* (WSP #48901).

[7] WSP numbers are accession numbers for the Mycological Herbarium, Department of Plant Pathology, Washington State University. Portions of the paratypes have been deposited in the National Fungus Collections and the Farlow Herbarium.

The fungus has been found occurring on potatoes, egg plant, and nightshade in the Yakima River Valley, Washington, U.S.A., from the vicinity of Prosser, Benton County, to Kittitas, Kittitas County.

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NOTES AND BRIEF ARTICLES

Tetrapolarity in Schizophyllum fasciatum

The recent acquisition of a collection of *Schizophyllum fasciatum* Pat. from Mexico has permitted the extension of our study of sexuality in *Schizophyllum* to a third described species. Collected in Nuevo Leon Province in September 1959 by P. A. Lemke, the material was sent to A. H. Welden, of Tulane University, who turned over half of the collection to Wm. Bridge Cooke. Cooke, who has generously served as taxonomic collaborator in our previous work on *Schizophyllum*, confirmed the identification of the collection and made it available for the present analysis.

Two small fruit bodies were initially sporulated, and a sample of monosporous progeny was collected. Preliminary matings among the strains of this sample revealed numerous dikaryotic mycelia, most of which produced sporulating fruit bodies by the tenth day after mating (Fig. 1). The pattern of mating within this initial sample suggested tetrapolarity, but the pattern was somewhat confused, probably as the result of different sets of incompatibility factors in the two fruit bodies. Another sample of spores was accordingly collected from a fruit body produced on one of the newly established dikaryons. Sixty-three strains of this sample were mated with each of the two parental strains. About one quarter of the sample was compatible with each parental strain, and abnormal hyphae like those of the common-*A* heterokaryon of *S. commune* were observed in many of the incompatible matings. By means of the latter, two nonparental mating types were provisionally identified, and these were mated with all strains of the sample that were incompatible with the parental strains. The results confirmed the occurrence of four distinct mating types and the segregative pattern of tetrapolar sexuality. A single strain, compatible with two of the four mating types, carried a nonparental factor of the *A* series and probably represented an intra-*A* factor recombinant. The distribution of mating types in the sample fits the expectation of tetrapolar segregation ($X^2 = 3.10$, $P = 0.4$):

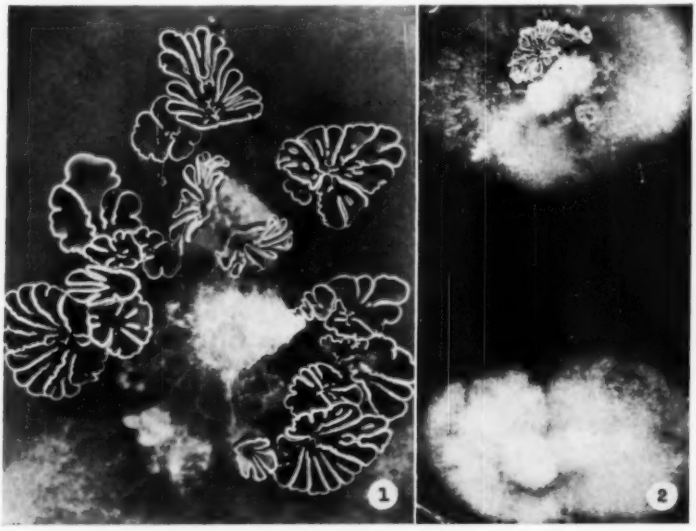
A^1B^1	A^1B^2	A^2B^1	A^2B^2	A^xB^x
16	21	12	13	1.

The incompatibility factor constitutions of the remaining five fruit bodies of the collection were also assayed. In the entire collection, there were at least five distinct *A* factors and four distinct *B* factors; these were distributed as follows:

Fruit bodies

(1 + 2)	3	4	5	6	7
$A^1A^2B^1B^2$	$A^3A^6B^1B^3$	$A^3A^4B^3B^4$	$A^1A^5B^1B^3$	$A^3A^4B^1B^3$	$A^3A^5B^1B^3$.

S. fasciatum is thus revealed as a typical tetrapolar species having multiple factors in both *A* and *B* series. The single nonparental *A* factor that was observed further indicates a compound structure of the *A* factor like that of *S. commune* (Raper, Baxter, and Middleton, **1958**).



FIGS. 1, 2. *Schizophyllum fasciatum* Pat. FIG. 1. Ventral aspect of fruit bodies at 10 days. × 3. FIG. 2. Compatible (above) and common-A matings at 7 days. × 1.

A few other observations are of interest. Culturally, the species is easily handled. Spore germination in all cases was >95%, and homokaryotic and dikaryotic mycelia grew rapidly and well on the various media developed for the study of *S. commune*. Homokaryotic and dikaryotic mycelia closely resemble those of *S. commune*—both microscopically and macroscopically.

The four types of mycelial interactions, compatible, common-*A*, common-*B*, and common-*AB*, also resemble those of *S. commune*, although they tend to be somewhat less distinctive macroscopically (FIG. 2). Furthermore, the common-*A* heterokaryon appears to be unstable —the hyphal morphology characteristic of the heterokaryon is transient —and the diagnostic value of the reaction is accordingly limited.

One of the two fruit bodies that were originally examined carried a biochemical deficiency, *adenineless*, which segregated normally (1:1) in a cross with a biochemically wild-type strain. A mutation to *cholineless* was recovered following irradiation with ultraviolet light.

Matings between biochemically wild strains of *S. fasciatum* and *S. commune* on complete medium resulted in no discernible interaction. Interspecific matings with massive inocula of biochemically deficient strains on minimal medium resulted in slow but continued growth; neither isolated hyphal tips nor small blocks of agar containing large numbers of hyphae yielded any appreciable growth, however, when transferred to fresh minimal medium. Given the gentle choice of interaction or starvation, they starved. There is thus no evidence of interspecific heterokaryosis—sexual or asexual.

Homokaryotic mycelia of *S. fasciatum* appear to be somewhat more stable in continued culture than are monosporous mycelia of *S. umbrinum*, which rapidly degenerate (Raper, **1959**). Some degeneration, presumably mutative as in *S. commune*, of a number of monosporous strains has been observed, however, and the maintenance of such strains for protracted periods would probably present the same difficulties experienced with *S. commune*. Because of the greater cultural stability of the genetically balanced dikaryotic mycelium, two dikaryons of *S. fasciatum* of the genotypes (A^1B^1 *adenineless* + A^2B^2 *adenineless*) and ($A^3B^3 + A^4B^4$) are being deposited in the National Type Culture Collection, Washington, and in the Centraalbureau voor Schimmelcultures, Baarn, Holland.

It is a pleasure to express our indebtedness to Wm. Bridge Cooke for these materials and for his continuing interest and participation in the study of sexuality in the genus, *Schizophyllum*.—JOHN R. RAPER, Harvard University.

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PLUTEUS LILACINUS

A revision of the type material of *Agaricus lilacinus* Mont. at Paris permits the identification of this species. I had already stated that it belongs in the *Cervinus*-group and believed then that it might possibly correspond to a small-spored form not otherwise identifiable. The revision of some additional sections from the holotype shows that on some gill edges the cheilocystidia are very well preserved and that they are all the elongate narrow type as in *P. magnus*. Furthermore, as had been anticipated, the hyphae are without clamp connections. The metuloids are more variable than might have been assumed from the first sample taken.

The type is therefore identical with a form I mentioned (Brit. Mycol. Soc. Trans. **39**: 175. 1956) and which I compared with *P. australis* Murr. Even if this tentative determination should be correct, Montagne's epithet has considerable priority. There is no trace of the color suggested by Montagne's name and mentioned by him in his original diagnosis, undoubtedly on the basis of erroneous notes or sketches sent by Sullivant. But since there is no other element in the type material, we have to follow Art. 72 of the International Code of Botanical Nomenclature and accept the epithet "lilacinus" as validly published, and nomenclatorially correct.

Pluteus lilacinus (Mont.) Sing. comb. nov.

Agaricus lilacinus Mont., Syll. Crypt. p. 110. 1856.
Mycena lilacina (Mont.) Sacc., Syll. Fung. **5**: 257. 1887.
?*Pluteus australis* Murr., Proc. Fla. Acad. Sci. **7**: 119. 1945.

Pileus brown in the color of *P. cervinus*, much paler on margin, smooth, glabrous, subviscid, 20–29 mm broad. Lamellae white, later sordid stramineous pink, broad, to moderately broad, close, free. Stipe white, smooth, glabrous, stuffed, or solid, tapering upwards or equal, mostly (in the Southern form) elongated but varying: 20–83 × 3–5.5 mm. Context white, inodorous, unchangeable, soft.

Spores 5–7.2 × 3.5–5.7 μ, mostly 5.5–7 × 4–4.2 μ, ellipsoid to cylindric, smooth, pale rosy stramineous. Hymenium consisting of metuloids, basidioles, basidia, and cheiolocystidia. Basidia 19–20 × 6–7 μ, 4-spored. Cheilocystidia of one single kind: elongate, 40–75 × 5.5–8.8(–12) μ, hyaline, smooth, numerous and making the edge heteromorphous, mixed with a few entire (*Magnus*-type) metuloids, cylindrical or cylindrical-subclavate. Metuloids mainly of the *Magnus*-type, 42–70 × 9.5–16 μ, with moderately but distinctly thickened hyaline wall and with colorless cell sap, generally fusoid and thickest either just below, at, or just above

the middle, mostly either short acute-pronged (to 4 prongs) at apex, or with sharply acute apex, very rarely (near edge) with obtuse narrow apex, sometimes with 1, very rarely 2 lateral spinules, numerous although not crowded; further away from edge also some metuloids which approach the *Cervinus*-type, i.e., ventricose, broader above than the other type, with up to 4 obtuse or acute prongs up to 7 μ long, 48–56 × 13.5–18 μ, wall 1.5–2 μ thick. Epicutis consisting of mostly filamentous relatively thin hyphae appressed and radially arranged, hyaline, fewer brownish, filled with a homogeneous pigment solution, with rounded ends. Hyphae without clamp connections.

On fallen rotting wood or on wood buried in humus (*Quercus* sp.), solitary or in small groups, fruiting from April until October.

Material studied: Florida (U.S.A.): Newnan's Lake, April 19, 1943, *R. Singer* F 1859 (F).—Ohio: Near Columbus, Sullivant no. 90 (icon.), July, (P, HOLOTYPE collection).

In order to determine this species correctly, the key published by me in 1956 (loc. cit., p. 223) should be modified so as to allow for more or less dimorphic metuloids under "M" below. The species will then key out with "*P. ? australis*" which should be corrected in *P. lilacinus*. —ROLF SINGER, Instituto M. Lillo, Tucumán, Argentina.

THE RELATION BETWEEN BICARBONATE, GLUCOSAMINE SYNTHETASE AND CHITIN SYNTHESIS IN BLASTOCLADIELLA

The bicarbonate trigger mechanism in the water mold, *B. emersonii* (Cantino and Turian, **1959**), leads to the formation of a resistant sporangium (R.S.) with a thick, chitinous wall. At the organismal level, the response is essentially all-or-none in character; that is, below 4×10^{-3} M, $NaHCO_3$ has no effect on R.S. morphogenesis, while at ca. 10^{-2} M, it induces essentially all plants in a population to form R.S. in which the walls are of uniform and normal thickness (Cantino, **1956**). Glucosamine synthetase (glutamine-fructose-6-phosphate transamidase), a relatively labile enzyme presumably involved in the *in vivo* synthesis of chitin, has been demonstrated in R.S. plants and purified ca. 20-fold (Lovett and Cantino, **1960**).

Our working hypothesis has been that bicarbonate, once it reaches a threshold level within the plant, causes rechanneling of essential building blocks in the direction of a functional, pre-existing biosynthetic chain leading to chitin synthesis. We felt that this notion of a bicarbonate-induced shift in overall equilibrium would be strengthened if it could be shown: (1), that a further increase in bicarbonate (beyond the optimal

level needed for differentiation of a normally-thick wall) would induce formation of an abnormally-thick wall; (2), that such an abnormal thickening was actually due to deposition of chitin; and (3), that key enzymes such as glucosamine synthetase increased in total quantity or specific activity, or both. The following results have been obtained.

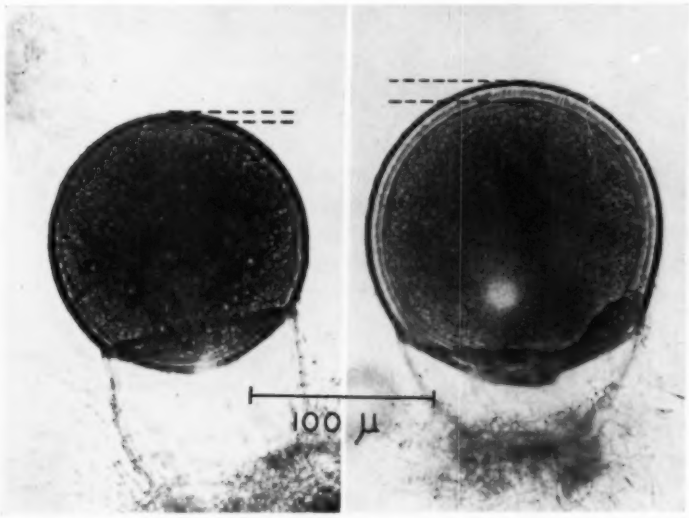

FIG. 1. RS plants of *Blastocladiella* grown in liquid medium PYG containing 8.9×10^{-3} M $NaHCO_3$ (left) and 2.38×10^{-2} M $NaHCO_3$ (right).

While 8.9×10^{-3} M $NaHCO_3$ (in Difco Cantino PYG Broth) induces 100% formation of normally-thick walled, pigmented, pitted R.S. plants (FIG. 1, left), super-optimal concentrations of $NaHCO_3$ (2.38×10^{-2} M) overdrive the metabolic machinery of the plant to produce a pronounced, abnormal thickening of the walls (FIG. 1, right). Corroborative analyses show that chitin deposition has, indeed, been increased almost two-fold (TABLE 1). Assays for glucosamine synthetase

TABLE 1

THE CHITIN CONTENT OF RS PLANTS GROWN ON OPTIMAL AND SUPEROPTIMAL CONCENTRATIONS OF BICARBONATE

Bicarbonate concn in medium PYG	μg chitin/mg dry wt of plant material*
8.93×10^{-3} M	92.5
2.38×10^{-2} M	160.3

* Determined according to Hackman (Austral. Jour. Biol. Sci. 7: 168, 1954) as modified by Lovett and Cantino (1960).

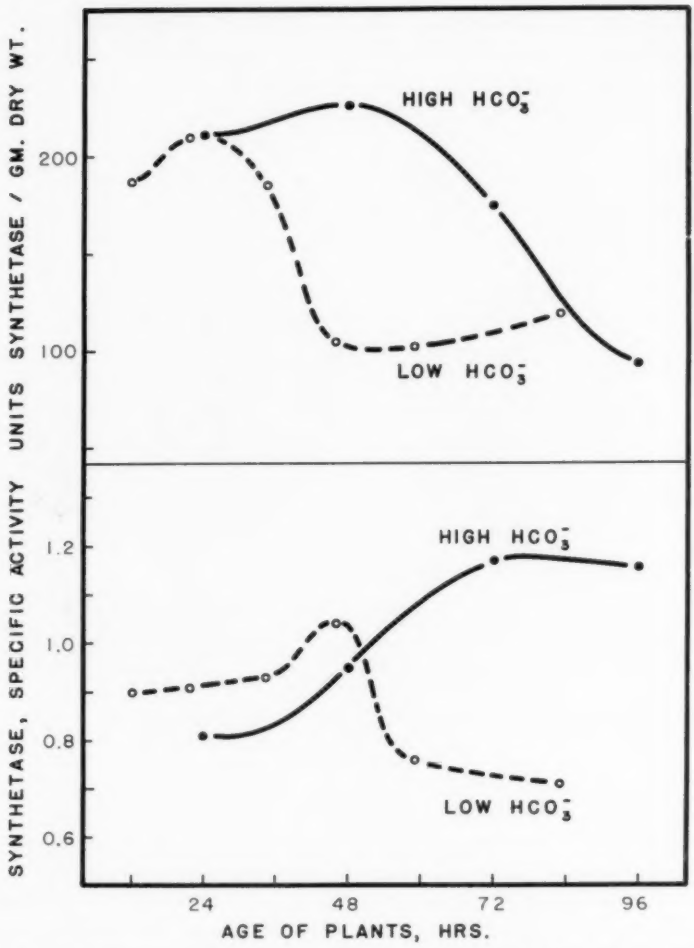

FIG. 2. Glucosamine synthetase in *Blastocladiella* during differentiation. Top: total units glucosamine synthetase/unit wt of organisms. One unit synthetase: the quantity of enzyme mediating production of 1 μM glucosamine-6-phosphate/20 min in the standard assay. Bottom: specific activity of glucosamine synthetase. Standard assay: 22,000 × G supernatants (contg. 0.6 mg sol. protein/assay) incubated with 20 μM glutamine, 20 μM fructose-6-phosphate, and 100 μM phosphate, pH 6.5, final vol of 2.0 ml, for 20 min at 30° C. The reaction was stopped with 7% Na_2WO_4 (0.01 ml) and 1N HCl (0.06 ml) and analyzed for glucosamine-6-phosphate by the Dische and Borenfreund method (Jour. Biol. Chem. **184**: 517. 1950). All values corrected for unincubated controls. Specific activity: μM glucosamine-6-phosphate/mg protein for 20 min.

activity at different stages in the development of R.S. plants in synchronous single-generation cultures (Lovett and Cantino, **1960**) reveal that the peak in total activity of glucosamine synthetase/unit weight of organism during ontogeny is delayed about 24 hours by the 2.7-fold increase in $NaHCO_3$ (FIG. 2, top). Microscopically, the morphological changes associated with R.S. development are also shifted by the same amount. Thus, when comparisons are made at corresponding morphological stages in development, the total amount of glucosamine synthetase/unit weight of organism grown on the elevated $NaHCO_3$ concentrations varies from 10 to 70% higher than that present at the lower bicarbonate levels; the final specific activities of the enzyme are greater by an equivalent margin (FIG. 2, bottom).

These experiments do not provide evidence for the mechanism which links carboxylation (cf. Cantino and Turian, **1959**) to chitin synthesis. But, they show that increased bicarbonate levels induce an increase in synthesis of chitin, as well as an increase in the specific activity of, and a prolonged retention of total activity of a key enzyme, glucosamine synthetase, presumably involved in the *in vivo* manufacture of the cell wall.

Our work was supported by an N.S.F. Fellowship to the first author and research grants from the National Science Foundation and the National Institutes of Health to the second author.—JAMES S. LOVETT AND EDWARD C. CANTINO, Department of Botany and Plant Pathology, Michigan State University, East Lansing, Mich.

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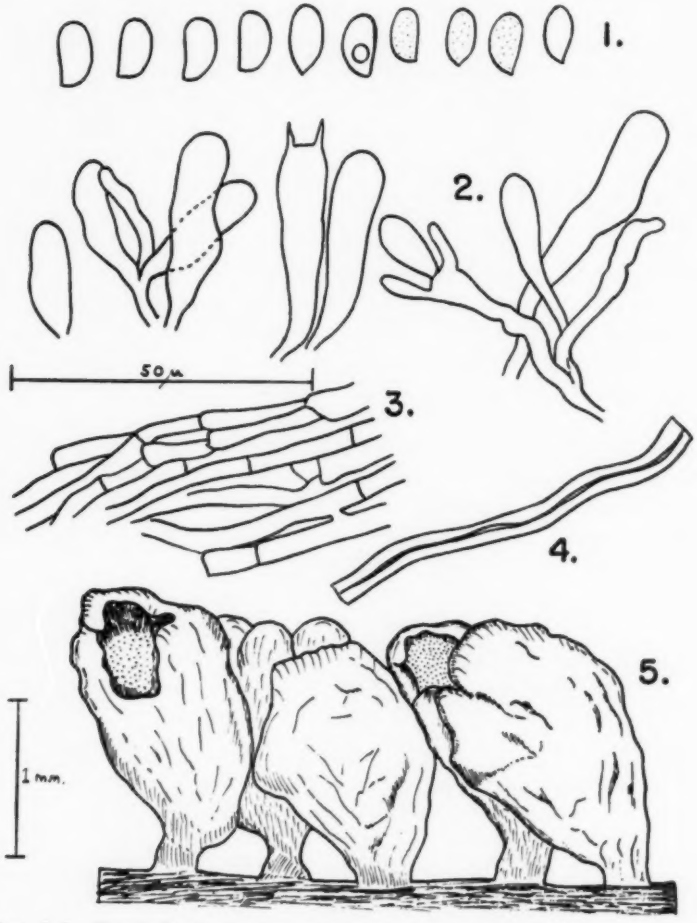
CALYPTELLA CAPENSIS FROM SOUTH AFRICA

After the publication of a survey of "The Cyphelloid Fungi of South Africa" by P. H. B. Talbot (Bothalia **6**: 465–487. 1956), a specimen of a cyphellaceous fungus was submitted to him for identification. It was forwarded to Cincinnati for further study after notes and drawings were made. It was found to be sufficiently different from other species

of *Calyptella* to merit separate attention preceding the publication of a monograph on the cyphelloid fungi. The fungus is a good representative of the genus *Calyptella* Quélet as typified by *Cyphella capula* Holmsk. ex Fr.



FIGS. 1–5. FIG. 1. Basidiospores. FIG. 2. Basidia. FIG. 3. Cuticle of brown hyphae. FIG. 4. Portion of a surface hair. FIG. 5. Habit of the receptacles.

Calyptella capensis W. B. Cooke and P. H. B. Talbot, sp. nov.

Receptacula subsessilia vel substipitata, alba, gregaria; stipa 0.3 mm diam et longit; cupulis infundibuliformibus cornucopioideformibus, 1.6–2.3 × 1.0 mm—extus

albis ad cinereas; cuticula cellulis 10–30 × 3–4 μ, pallide brunnea, piliis 100 × 3–4.5 μ, hyalinis, tortuosis, non spiralibus munita; hymenio pallide luteo; basidiis 30–40 × 7.4–8.3 μ, clavatis, 2–4-sterigmatibus; sporis ellipsoideis, hyalinis, laevibus vel minute punctatis, apiculatis, 9.3–10.6 × 4.5–5.5 μ.

Receptacles subsessile to substipitate, pendent to gregarious, tending to cohere with each other above, white when fresh; stipe to 0.3 mm in diameter and 0.3 mm long; cupules narrowly funnel-shaped, 1.6–2.3 mm tall, 1 mm broad; surface white to gray, formed of a cuticle from which arise surface hairs; cuticle formed of elongated cells 10–20 × 3–4 μ, light brown in color, thin-walled, with H-pieces; surface hairs 3–4.5 μ in diameter, up to 100 μ long, hyaline, thick-walled, with little or no lumen, undulate to zig-zag, not spirally twisted; hymenium smooth, light yellow, formed of a tightly packed palisade of basidia; basidia 30–40 × 7.4–8.3 μ, clavate, 2–4-sterigmate; spores oblong-ellipsoid, flattened on one side, hyaline, smooth, minutely punctate under the oil immersion objective, apiculate, (7.9–)9.3–10.6 × (4.2–)4.5–5.5 μ; no clamp connections observed.

Habitat: On litter of forest floor.

TYPE and specimen examined: Hogsback, Cape Province, Union of South Africa. Collected April 14, 1957 by *A. R. A. Noel.* Rhodes University No. 10120. (PRE 41806, WBC.)

This species differs from other light colored species of *Calyptella* with relatively large spores by the absence of clamp connections. *C. capula,* of wide distribution, has acanthophyses on the surface of the receptacles in many specimens: *Cyphella cernua* (Schum.) Massee, of which specimens have been seen from England and Italy, has surface dichophyses; while *C. hebe* Cunningham, from New Zealand, has longer spores which reach 12–13 μ in length. Specimens of those species which have been observed are campanulate to funnel-shaped. The figures were furnished by Dr. Talbot.—WM. BRIDGE COOKE, Robert A. Taft Sanitary Engineering Center, Cincinnati 26, Ohio.

A NOTE ON UROCYSTIS CEPULAE

From the time of Winter's classification in Rabenhorst's Kryptogamen Flora (1884), which listed *Urocystis cepulae* Frost as a synonym of *U. colchici* (Schlecht.) Rabenhorst, until Fischer's Manual of the North American Smut Fungi (1953) there has been argumentation regarding the possible synonymy. Naturally the answer depends somewhat upon the definition used in separating fungus species. In my opinion there are enough differences between the two organisms to consider them as separable.

The first dissimilarity is that the two pathogens cannot be cross inoculated successfully. This statement has been confirmed by other authors and by the fact that *U. colchici* was present in Europe long before the onion fungus appeared there, while *U. cepulae* was found in the United States many years before *U. colchici* was discovered in this country.

The spores, too, are distinct in size, shape, and formation (FIG. 1). Without looking at the slide label it is easy to name the species when successive slides of the two fungi are placed at random under the microscope. *U. cepulae* spores from Long Island, upstate New York, and

FIG. 1. Spores of *Urocystis colchici* (left) and *U. cepulae* (right). Drawing by Miss A. Abbe.

Maine; and those of *U. colchici* from Germany, Czechoslovakia, and Long Island were mounted semi-permanently and examined in the above manner.

The measurements of 20 spores from each slide gave the following dimensions:

	Inner spore cavity	Entire spore
From onion	10–16 microns	15–22 microns
From colchicine	12–24	17–40

Fresh spores of each would probably be slightly larger.

The onion pathogen has mostly one inner spore and only rarely two. An attempt was made to determine the exact percentage with the one inner spore. This was made difficult because sometimes two or more spores clung together. The percentage appeared to be in the eighties. On the other hand, *U. colchici* was mostly multiple spored, and sometimes as many as four spores occurred in one cluster.

The jacket cells that surround each spore or spore cluster also are distinctive in the two pathogens. The cells of *U. cepulae* are uniform in shape and size, in optical section usually 12 to 15 in number, and subhyaline to very faintly colored. Those of *U. colchici* are large, irregular in shape, and often no more than 6 or 9 in number. Although pale, they plainly are colored. These jacket cells make it easy to recognize each of the two organisms.

If we accept the rule that any two fungi incapable of being cross inoculated and which can readily be differentiated in culture or under the

microscope are distinct species, then the above two fungi should not be placed in synonymy.—CHARLES CHUPP, Cornell University, Ithaca, New York.

A NEW SPECIES OF CERCOSPORA ON ACER SACCHARINUM

Browning and dropping of leaves of several soft maple trees, *Acer saccharinum* L., in Metropolis, Illinois, were brought to the attention of the junior author by Mr. Dean Rodgers on October 15, 1959. Mr. Rodgers reported that some of these trees had lost most of their foliage early in the fall, although no frost had occurred. One tree that had not become severely defoliated was examined and several discolored leaves were collected.

Microscopic examination revealed a species of *Cercospora* on the under surface of the necrotic areas of these leaves. This *Cercospora*, found to be distinctly different from previously reported species on maple, is described as a new species.

Chupp [1] considered *Cercospora acericola* Woronichin and *C. negundinis* Ellis & Everhart to be true members of this genus and pointed out that *C. acerina* var. *tatarici* Garbowski may or may not be a true *Cercospora*. A brief comparison shows that this new species differs from *C. acericola* by producing narrower conidia with rounded to truncate bases and septate conidiophores with swollen bases. It differs from *C. negundinis* by producing conidia that are narrower and conidiophores that are mostly two-septate. The new species differs from *C. acerina* var. *tatarici*, as described by Garbowski,[2] by producing longer, slightly narrower conidia and longer, wider conidiophores.

Cercospora saccharini sp. nov.

Maculis angularibus, canescenti-brunneis, 1–1.5 mm latis, crescentibus in areolis magnis irregularibus; caespitulis hypophyllis; stromatibus fere globosis, brunneis, 30–45 μ diam; conidiophoris fasciculatis, omnino dilute olivaceo-brunneis, non ramosis, leviter geniculatis, ad basim tumidis, 1–3-septatis, 22–54 × 5–7 μ; conidiis hyalinis, rectis vel curvatis, 3–9-septatis, aciculatis vel paene cylindraceis, ad basim obtusatis vel truncatis, apicibus subobtusis, (38–)54–81(–124) × 3–3.5 μ.

Leaf spots angular, grayish brown, 1–1.5 mm wide, becoming large irregular blotches; fruiting hypophyllous; stromata mostly globose,

[1] Chupp, C. 1953. A monograph of the fungus genus *Cercospora*. Published by the author, Ithaca, N. Y., 667 p.

[2] Garbowski, L. 1923. Les micromycètes de la Crimée et des districts limitrophes de la Russie méridionale en consideration spéciale des parasites des arbres et des arbrisseaux fruitiers. Bull. Soc. Mycol. Fr. **39**: 227–260.

brown, 30–45 μ in diameter; conidiophores fasciculate, light olivaceous brown, fairly uniform in color, not branched, slightly geniculate, with swollen base, 1–3-septate (mostly two-septate), 22–54 × 5–7 μ; conidia hyaline, straight or curved, 3–9-septate, acicular to almost cylindric, base rounded to truncate, tip subobtuse, (38–)54–81(–124) × 3–3.5 μ.

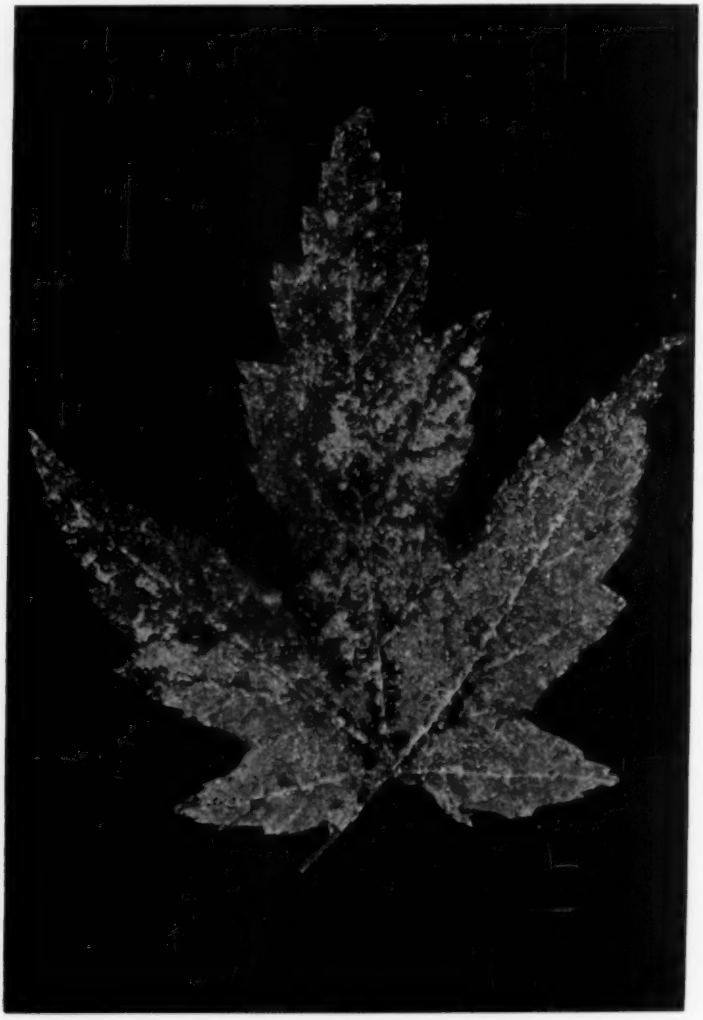


FIG. 1. Upper surface of leaf (*Acer saccharinum*) showing dark necrotic areas.

SPECIMENS EXAMINED: On *Acer saccharinum* L., Metropolis, Illinois, Oct. 15, 1959. Ill. Nat. Hist. Survey accession number 33219, TYPE.

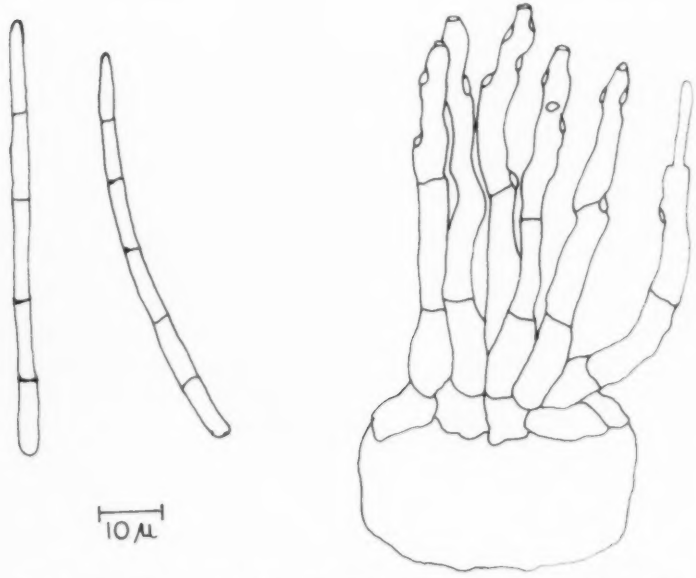


FIG. 2. *Cercospora saccharini.* Camera lucida drawings of conidia and conidiophores on outlined stroma. Conidiophore on the right bears a developing conidium.

A specimen has also been deposited in the herbarium of Mycological Collections, Bureau of Plant Industry, Beltsville, Maryland.—ANTHONY E. LIBERTA and G. H. BOEWE, Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Urbana, Illinois.

SUPPRESSION OF FUNGI BY LIGHT ON MEDIA CONTAINING ROSE BENGAL [1, 2]

In recent years, media containing the fluorescent dye rose bengal and antibiotics have been used increasingly in the isolation of fungi (**4**, **5**). Martin (**6**) and Farrow (**2**) used a rose bengal-streptomycin (RBS)

[1] Contribution No. 561, Kansas Agricultural Experiment Station, Manhattan, Kansas. Botany serial No. 721.

[2] This work was carried out under U. S. Public Health Grant E-1380 C (3).

medium in isolating soil fungi, and Cooke (**1**) a rose bengal-aureomycin (RBA) medium in isolating fungi from sewage. RBS was found best for isolating fungi from air in Kansas (**9**). This medium has several advantages when used in mycological or plant pathological work. Bacteria are completely inhibited, and lateral growth of fungi is reduced so that colonies remain small, permitting accurate counts. Even such spreaders as *Rhizopus* are so reduced that colony size may be only 2–3 cm after 7 days. Because of these excellent qualities, it will undoubtedly be used by more and more workers. However, in the early phases of work now in progress on the diurnal periodicity of fungi in the air, occasionally inconsistent results were obtained during midday. This has been found to be due to photodynamic changes of the dye that inhibit or suppress colony formation. This paper draws attention to the effects that light has on the growth of fungus colonies on this medium.

In 1957 work was initiated on the numbers and kinds of fungi present in the air during a 24-hour period, using RBS agar (**2**) for colonies and silicone slides for spore numbers with exposures being made in a slit sampler (**8**) on the roof of a campus building. In many of the series, negative plates were obtained during midday, yet spore numbers did not show a corresponding drop. When other media were used, such as potato dextrose agar (PDA), no decline in numbers was observed. This indicated that the RBS medium was responsible for the drop in numbers, since the plates had not been shielded from sunlight. The following experiments were then performed.

On July 23, 1959, a series of plates, PDA, RBS, and S (which lacked rose bengal) was exposed in the slit sampler 1 minute, then placed in the sun 20 minutes. All plates were kept in a light-tight box before and after exposure. Plates were incubated in the dark for 7 days at 72° F. The results are given in TABLE 1. It is evident that the medium containing rose bengal had been altered by exposure to the sun in such a way that no fungi were able to develop.

In another test (August 5, 1959), RBS plates were placed in the sun both before and after exposure in the slit sampler. Fungus colonies did not develop in either case whereas controls had normal numbers.

Since the concentration of rose bengal in RBA medium (350 mg/liter) is greater than in the RBS medium (50 mg/liter) an experiment was performed in which these two media were exposed for varying periods of time at 6:00, 8:00, 11:00 A.M. and 1:00 P.M. After a 1-minute exposure in the sampler, all plates except controls were removed from the light-tight box and placed in the sun for 3 to 21 minutes. At

6:00 A.M. there was no decrease in colonies with exposure to the sun, the variation in numbers being typical of air sampling. At 8:00 A.M., with increased radiation, colonies on RBA dropped rather sharply after 12 minutes exposure, while in RBS the decline was gradual. At 11:00 A.M., with greater radiation and higher temperatures, both media showed a pronounced inhibitory effect with negative plates after 6 minutes exposure. Similar results were obtained at 1:00 P.M. In all cases the RBA medium was more sensitive to light than the RBS medium, but both media were drastically altered by even a short exposure to strong sunlight.

TABLE 1

EFFECT OF SUNLIGHT ON MEDIA AT 8:50–10:00 AM JULY 23, 1959, FROM ROOF OF CAMPUS BUILDING, KANSAS STATE UNIVERSITY, MANHATTAN, KANSAS, FOLLOWING 1 MINUTE EXPOSURE IN A SLIT SAMPLER

Media*	Treatment	Plate					
		1	2	3	4	5	6
		Total number of fungus colonies					
PDA	In sun 20 min.	87	94	89	107	178	71
RBS	In sun 20 min.	0	0	0	0	0	0
S	In sun 20 min.	84	108	89	98	78	57
RBS	In darkness 20 min.	73	79	76	164	46	67

* PDA = potato dextrose agar, RBS = rose bengal-streptomycin, S = same as RBS but rose bengal omitted.

Climatic data: Relative humidity 41%; temperature 80°; sun temperature 106°; total radiation for sampling period 54.0 (Langley units).

The bacteriostatic action of light on fluorescein dyes in gelatin was reported by Mettler (**7**) in 1905, and the photodynamic action is apparently well known to bacteriologists. However, very few studies have been made on the fungistatic action of light on media containing these dyes. Freeman and Giese (**3**) have recently reported photodynamic action in a medium containing rose bengal in which cell growth of the yeast *Saccharomyces cerevisiae* was inhibited when exposed to light. Because of the many advantages of the rose bengal medium, it is extremely valuable for isolation of fungi from pathological material, soil, sewage, and other heavily contaminated sources. It must, however, be used with care since accidental exposure to strong light could result in misleading or even negative results. Fortunately, the diffuse light of ordinary laboratory illumination seems to have no appreciable effect.—S. M. PADY, C. L. KRAMER, AND V. K. PATHAK, Department of Botany and Plant Pathology, Kansas State University, Manhattan, Kansas.

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REVIEWS

DEMATIACEOUS HYPHOMYCETES: II, by M. B. Ellis. 23 p., 15 figs. Mycological Papers, No. 79. Commonwealth Mycological Institute, Kew. January, 1961. *7s.*

Earlier major papers of M. B. Ellis (*vide* Mycol. Papers Nos. 56, 65, 67, 69, 70, and 72. 1953–1959.) were devoted to hyphomycetous genera with relatively large numbers of species which required semi-monographic treatment, *e.g., Periconia, Corynespora, Clasterosporium.* This second paper of a new series (*vide* Mycol. Papers No. 76. 1960.) continues the proposal "to include genera which have only a small number of species as well as some with many species about which much confusion exists at the present time."

These taxonomic studies are proving to be excellent vehicles for the redescription of classic genera which have been misunderstood, forgotten, or ignored. They follow the admirable procedures now standard at C.M.I.: full "standardized" descriptions, carefully executed figures emphasizing the developmental morphology of the conidial apparatus, and keys to species. New genera are an additional feature of these studies. This latest paper contains reworking of *Septosporium* Corda and *Balanium* Wallroth and the establishment of a new genus with 10 species, *Acrodictys* M. B. Ellis.—EMORY G. SIMMONS.

THE HALLUCINOGENIC FUNGI OF MEXICO: AN INQUIRY INTO THE ORIGINS OF THE RELIGIOUS IDEA AMONG PRIMITIVE PEOPLES, by R. Gordon Wasson. Botanical Museum Leaflets [Harvard University] **19**: 137–162. 17 Feb. 1961.

Mr. Wasson presented this paper as the Eleventh Annual Lecture of the Mycological Society of America, Stillwater, Oklahoma, August 30, 1960. An appendix enumerates the known species of hallucinogenic mushrooms of Mexico and indicates for each species the earliest report of its use in Mexico as a divinitory agent.

A KÁRPÁT-MEDENCE ÚSZÖGGOMBÁI (SMUTS OF THE CARPATHIAN BASIN), by Gusztáv Moesz. 255 p., 102 plates. Egyetemi Könyvkiadó N.V., Budapest. 1950. Price, 48.50 Forint (about $4.50).

This monograph, a posthumous work of Dr. Gusztáv Moesz, one of Hungary's eminent mycologists, was completed in 1944 but was delayed in publication until after the war when Dr. G. Ubrizsy edited the manuscript.

In a comprehensive introductory essay of 46 pages there is a discussion of sexuality in the Ustilaginales, types of infection, control methods, a historical survey of important contributions to the knowledge of the smuts by Hungarian authors and other related topics. There are keys to the 17 genera and 127 species treated. The author has illustrated the text with generally excellent drawings of host plants, spores and other pertinent microscopic details. The genera *Ustilago* (44 spp.), *Entyloma* (18 spp.), *Urocystis* (18 spp.), *Cintractia* (9 spp.) and *Sphacelotheca* (8 spp.) account for nearly 75% of the species reported. For each species there is given a synonymy, description and commentary, distribution data, a list of herbaria consulted and literature citations. The systematic presentation is followed by a host index, a bibliography of 155 titles and a species index. The work concludes with a French translation of that part of the introduction dealing with historical aspects of the study. While the French version makes this section comprehensible to most mycologists, the rest of the Hungarian text is equally worthy of consideration. An English edition would provide many more mycologists with a valuable, authoritative source of information. It might also serve to increase our awareness of important contributions sometimes overlooked because of the language barrier.—B. LOWY.

ILLUSTRATED GENERA OF WOOD DECAY FUNGI, by Charles L. Fergus. vi + 132 p., 81 figs. Burgess Publishing Co., Minneapolis, Minnesota. 1960. Price $4.00.

This is an expansion and revision of keys and illustrations of wood-decay Hymenomycetes developed by the late L. O. Overholts for a course in forest pathology. An introduction explains the use of keys and gives directions for collecting fungi. A key to the families and a key to the 85 genera treated follow.

The detailed treatments are arranged mostly in the sequence of families familiar from Engler and Prantl, and genera are again keyed out in each family. Each genus is fully described, and the important species briefly discussed, with keys to the species in several larger genera. In the Hydnaceae and Polyporaceae a comprehensive key to species is given. At least one representative species is illustrated for 81 genera, supplemented, for many, by line drawings of the diagnostic microscopic

characters. A list of general references, a glossary of over 160 terms, and indices complete the book.

The illustrations are almost always distinctive and are uniformly well-reproduced. The book should fulfill very usefully its purpose to enable a general forester to identify decay fungi, and to supplement the illustrations in general forest pathology texts.—J. L. LOWE.

ILLUSTRATED GENERA OF IMPERFECT FUNGI, Second Edition, by H. L. Barnett. iii + 225 p., 462 figs. Burgess Publishing Co., Minneapolis, Minnesota. 1960. Price $4.50.

This revision treats 462 genera, more than half again as many as in the first edition, although the text is only 7 pages longer. To accomplish this condensation the drawings are on a more reduced scale (without loss of detail), and the 301 references have been grouped at the end of the book rather than cited with each genus.

The very usable key to the genera, including more than one-third of those known, utilizes striking, readily observable characteristics and, within the orders, takes out genera without regard to family classification. In the descriptions and illustrations the generic arrangement gives weight, in decreasing scope, to color of hyphae and conidiophores, to septation of spores, and to the increasing complexity of the conidiophore. In each genus the description briefly states the diagnostic morphological characters, the nutritional relationships, and, often, the habitat. Each genus is illustrated, slightly over 200 original with the author, and most of the remainder redrawn from original sources. The references give access to an enormous mass of literature, and current literature is satisfactorily covered, as indicated by the more than 70 references dated 1956–1960.

The Imperfect Fungi are an enormous group of plants of great mycological interest and economic importance, long neglected but now receiving increasing attention in classroom and professional work. Identification of imperfects has been extremely difficult as the literature is scattered, often out-of-print, and inaccessible to most nonspecialists. Determination of these organisms by students will be greatly aided by the accurate illustrations which will assist in interpretating the often minor or variable characters used to separate the form genera. Dr. Barnett has performed a signal service in collecting this scattered information and presenting it in a comprehensive and very useful form.—J. L. LOWE.

BRITISH CUP FUNGI AND THEIR ALLIES. AN INTRODUCTION TO THE ASCOMYCETES, by R. W. G. Dennis. xxiv + 280 p., illus. The Ray Society, London. 1960. Price 80 shillings.

From a first glance at the title, one might presume that this work was chiefly concerned with the Discomycetes. A closer inspection, however, reveals that the "allies" mentioned in the title include the other Ascomycetes. Mycologists familiar with the author's earlier papers on the British Hyaloscyphaceae and Helotiaceae will welcome the appearance of this volume of much wider scope.

The classification followed is primarily that outlined by Nannfeldt in 1932, with modifications by Le Gal in the Pezizales and Luttrell, Munk, von Arx and Mueller in other groups. Under the Euascomycetes are included the Taphrinales, Pezizales, Tuberales, Plectascales, Ostropales, Clavicipitales, Lecanorales, Phacidiales, Helotiales, Coronophorales and Sphaeriales; under the Loculoascomycetes are the Myriangiales, Hysteriales, Hemisphaeriales, Dothideales (including Pseudosphaeriaceae) and Pleosporales. Since the book is intended, at least in part, to stimulate interest of amateurs in this group, the introduction describes briefly the chief morphological features of these fungi and gives suggestions on the collecting and preparation of specimens, as well as on staining techniques. The author's general observations on taxonomy and nomenclature will be of interest to all taxonomists.

Diagnostic keys to families and genera are included and the essential characters of each genus and species noted, with data on their occurrence in Great Britain. No new genera or species are described but fitting some species into the classification adopted has necessitated proposing about a dozen new combinations. Sixty plates, of which 40 are in color, illustrate nearly all of the genera. An annotated list is given of the more important books useful to the student of the Ascomycetes. This first comprehensive treatment of the British Ascomycetes to appear for many years will be extremely valuable, not only to those interested in the fungi of the region covered, but to mycologists in many other parts of the world.—EDITH K. CASH.

AFRICAN FUNGI. I., by F. C. Deighton. 43 p., 22 figs., 2 pls. Mycological Papers, No. 78. Commonwealth Mycological Institute, Kew. 28 September, 1960. 12*s*.

This paper initiates a series which the author states "will deal with miscellaneous fungi from Africa, chiefly from Sierra Leone, which are

represented in the herbarium of the Commonwealth Mycological Institute."

In this first paper, which deals exclusively with Hyphomycetes, 4 new genera, 11 new species, and 2 new combinations are proposed. The new genera are *Megalodochium, Helicominopsis, Hansfordiellopsis,* and *Ampullifera.* New species proposed are *Linotexis tympani-malleus, Hansfordia cinnamomi, Megalodochium palmicola, Helicominopsis fici, Sclerographium phyllanthicola, Trichothyrium asterolibertiae, Hansfordiella diedickiae, Hansfordiellopsis aburiensis, Ampullifera foliicola, A. ugandensis,* and *A. leonensis.* The new combinations are *Tretopileus sphaerophorus* (Berk. & Curt.) Hughes & Deighton (= *Monotospora sphaerophora* Berk. & Curt.) and *Memnoniella subsimplex* (Cooke) Deighton (= *Stachybotrys subsimplex* Cooke). In addition, the genus *Xylohypha* (Fries) Mason, which had not been validly published, is given valid publication, along with 3 combinations previously made into the genus.

Most of the species listed have been collected in Africa by the author. All are adequately illustrated by line drawings and described in detail from field collections.

This series on African fungi promises to be a valuable addition to the Mycological Papers of the C.M.I.—R. L. GILBERTSON.

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North American Flora. Descriptions of the wild plants of North America, including Greenland, the West Indies, and Central America. Planned to be completed in 34 volumes. Roy. 8vo. Each volume to consist of four or more parts. [Not offered in exchange.] Volumes 1–10 devoted to fungi.

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Vol. 2, part 1, 1917. Blastocladiaceae, Monoblepharidaceae, Saprolegniaceae, Ectrogellaceae, Leptomitaceae.

Vol. 3, part 1, 1910. Necrineae-Fimetariaceae.

Vol. 5, part 1, 1922. Phyllostictaceae (part).

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Vol. 9 (now complete), parts 1–7, 1907–1916. Polyporaceae-Agaricaceae (part).

Vol. 10, part 1, 1914; parts 2 and 3, 1917; part 4, 1924; part 5, 1932. Agaricaceae (part).

Series II, part 1, 1934. Tuberales.

Brittonia, quarterly; beginning in 1937, official publication of the American Society for Plant Taxonomists. $7.50 a year.

Economic Botany, quarterly; review and research articles dealing with plants useful to man; established 1947; beginning 1959, the official journal for The Society for Economic Botany. $4.00 a year.

The Memoirs of The New York Botanical Garden, issued irregularly beginning in 1900; approximately 500 pages per volume. $10.00 a volume.

The Myxomycetes of North America. By Robert Hagelstein. 1944. $7.50.

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